	L #	Hits	Search Text	DBs	Time Stamp
1	L1	4270	carbon near2 (flux or flow)	USPAT; US-PGPUB	2003/12/24 07:56
2	L2	275	1 near4 (modif$8 or alter$8 or increas$8)	USPAT; US-PGPUB	2003/12/24 07:56
3	L3	166	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT; US-PGPUB	2003/12/24 07:57
4	L4	9	2 and 3	USPAT; US-PGPUB	2003/12/24 07:57
5	L5	6159	phosphotransferase$1 or phospho adj transferase$1	USPAT; US-PGPUB	2003/12/24 08:10
6	L6	36	(2 or 3) and 5	USPAT; US-PGPUB	2003/12/24 08:11
7	L7	40	(2 or 3) same (aromatic or shikimate)	USPAT; US-PGPUB	2003/12/24 08:14

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	4270	carbon near2 (flux or flow)	USPAT; US-PGPUB	2003/12/24 07:56
2	L2	275	1 near4 (modif$8 or alter$8 or increas$8)	USPAT; US-PGPUB	2003/12/24 07:56
3	L3	166	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT; US-PGPUB	2003/12/24 07:57
4	L4	9	2 and 3	USPAT; US-PGPUB	2003/12/24 07:57

PGPUB-DOCUMENT-NUMBER: 20030134392

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030134392 A1

TITLE: Microorganisms and methods for overproduction of DAHP by cloned Pps gene

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 289788

DATE FILED: November 7, 2002

RELATED-US-APPL-DATA:

child 10289788 A1 20021107

parent division-of 09440503 19991115 US GRANTED

parent-patent 6489100 US

US-CL-CURRENT: 435/74, 435/252.33 , 435/320.1

ABSTRACT:

Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (15):

[0014] The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common aromatic pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

Summary of Invention Paragraph - BSTX (19):

[0017] The present invention further provides a method for **increasing carbon**

flow for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a pps gene so that Pps is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

Summary of Invention Paragraph - BSTX (20):

[0018] The present invention further provides methods of **increasing carbon flow** into the common aromatic pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

Detail Description Paragraph - DETX (3):

[0031] The inventor have found that cell lines can be developed that **increase the carbon flux** into DAHP production and achieve near theoretical yields of DAHP by overexpressing phosphoenolpyruvate synthase (Pps) in the cell lines. Overexpression of Pps can increase the final concentration and yield of DAHP by as much as two-fold, to a near theoretical maximum as compared to wild type cell lines. The overexpression of Pps is achieved by transforming a cell line with recombinant DNA comprising a pps gene so that Pps is expressed at enhanced level relative to the wild type cell line and so that the yield of DAHP approaches its theoretical value.

Detail Description Paragraph - DETX (5):

[0033] Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-aromatic, and aromatic metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common aromatic pathway relative to wild type host cells not containing the genetic elements of the present invention.

Detail Description Paragraph - DETX (6):

[0034] In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common aromatic pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps gene so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium where the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

Detail Description Paragraph - DETX (8):

[0036] Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

Detail Description Paragraph - DETX (12):

[0040] Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the phosphotransferase system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common aromatic pathway or pathways branching therefrom.

Detail Description Paragraph - DETX (15):

[0043] A key component of the methods of the present invention directed at **increased carbon flux** commitment to DAHP and DAHP metabolites is the recycling of pyruvate to PEP. Pyruvate is available in host cells as an end product of glycolysis.

Detail Description Paragraph - DETX (93):

[0119] This example demonstrates that the E. Coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pATI (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

Detail Description Paragraph - DETX (96):

[0122] To determine whether the Pps effect requires overexpressed transketolase (Tkt) as well, plasmid pRW5, which contains only aroG.sup.fbr,

was used in place of pATI in the above experiments. It was found that overproduction of Pps did not increase the DAHP production (FIG. 2A) without the elevated Tkt activity. Therefore, as limitation of small molecules in the biosynthesis of DAHP is concerned, the first limitation arises from the supply of E4P. This bottleneck shifts to the **supply of PEP** when Tkt is overexpressed, which is believed to increase the supply of E4P.

Detail Description Paragraph - DETX (99):

[0124] Since PEP is also converted to OAA by Ppc, the deletion of this enzyme may increase the **supply of PEP**. Therefore, the ppc gene on the chromosome of AB2847 was inactivated to determine whether DAHP production could be increased without Pps overexpression. This was done by transducing AB2847 with a P1 lysate grown on JCL1242 ppc::Km. The resulting transductant, JCL1283 aroB ppc::Km was then transformed with pATI or pRW5 and tested for DAHP production in the re-suspension culture as described above. To avoid limitation of OAA in the ppc strain, the culture medium was supplemented with succinate, which was shown to have no effect on DAHP production (data not shown). Contrary to the expectation, ppc mutation did not increase the production of DAHP (FIG. 2B), suggesting that the metabolic flux from PEP to OAA was not significant under the experimental conditions tested here. In fact, the ppc mutation actually decreased the DAHP production for unknown reasons.

PGPUB-DOCUMENT-NUMBER: 20030087381

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087381 A1

TITLE: Metabolically engineered organisms for enhanced production of oxaloacetate-derived biochemicals

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 215440

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

child 10215440 A1 20020809

parent continuation-in-part-of 09417557 19991013 US GRANTED

parent-patent 6455284 US

child 09417557 19991013 US

parent continuation-in-part-of PCT/US99/08014 19990413 US PENDING

non-provisional-of-provisional 60081598 19980413 US

non-provisional-of-provisional 60082850 19980423 US

US-CL-CURRENT: 435/69.1, 435/193 , 435/252.3 , 435/252.33 , 435/320.1 , 536/23.2

ABSTRACT:

Metabolic engineering is used to **increase the carbon flow** toward oxaloacetate to enhance production of bulk biochemicals, such as lysine and succinate, in industrial fermentations. Carbon flow is redirected by genetically engineering the cells to overexpress the enzyme pyruvate carboxylase.

[0001] This application is a continuation-in-part application of U.S. application Ser. No. 09/417,557, filed Oct. 13, 1999, which is a continuation-in-part of International Application PCT/US99/08014, with an international filing date of Apr. 13, 1999, which in turn claims the benefit

of U.S. Provisional Application No. 60/081,598, filed Apr. 13, 1998, and U.S. Provisional Application No. 60/082,850, filed Apr. 23, 1998, each of which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Metabolic engineering is used to **increase the carbon flow** toward oxaloacetate to enhance production of bulk biochemicals, such as lysine and succinate, in industrial fermentations. Carbon flow is redirected by genetically engineering the cells to overexpress the enzyme pyruvate carboxylase.

Summary of Invention Paragraph - BSTX (10):

[0010] Various metabolic engineering strategies have been pursued, with little success, in an effort to overcome the network rigidity that surrounds carbon metabolism. For example, overexpression of the native enzyme PEP carboxylase in E. coli was shown to **increase the carbon flux** towards oxaloacetate (C. Millard et al., Appl. Environ. Microbiol., 62, 1808-1810 (1996); W. Farmer et al, Appl. Env. Microbiol., 63, 3205-3210 (1997)); however, such genetic manipulations also cause a decrease in glucose uptake (P. Chao et al., Appl. Env. Microbiol., 59, 4261-4265 (1993)), since PEP is a required cosubstrate for glucose transport via the phosphotransferase system. An attempt to improve lysine biosynthesis in Corynebacterium glutamicum by overexpressing PEP carboxylase was likewise not successful (J. Cremer et al., Appl. Env. Microbiol., 57, 1746-1752 (1991)). In another approach to divert carbon flow toward oxaloacetate, the glyoxylate shunt in E. coli was derepressed by knocking out one of the transcriptional regulators, fadR. Only a slight increase in biochemicals derived from oxaloacetate was observed (W. Farmer et al., Appl. Environ. Microbiol., 63, 3205-3210 (1997)). In a different approach, malic enzyme from Ascaris suum was overproduced in mutant E. coli which were deficient for the enzymes that convert pyruvate to lactate, acetyl-CoA, and formate. This caused pyruvate to be converted to malate which increased succinate production (see FIG. 2). However, this approach is problematic, since the mutant strain in question cannot grow under the strict anaerobic conditions which are required for the optimal fermentation of glucose to organic acids (L. Stols et al., Appl. Biochem. Biotechnol., 63-65, 153-158 (1997)).

Detail Description Paragraph - DETX (95):

[0113] In many organisms PEP can be carboxylated to oxaloacetate via PEP carboxylase or it can be converted to pyruvate by pyruvate kinase (I. Shiio et al., J. Biochem., 48, 110-120 (1960);M. Jetten et al., Appl. Microbiol. Biotechnol., 41, 47-52 (1994)). One possible strategy that was tried to **increase the carbon flux** toward oxaloacetate in C. glutamicum was to block the carbon flux from PEP toward pyruvate (see FIG. 3). However, lysine production by pyruvate kinase mutants was 40% lower than by a parent strain, indicating that pyruvate is essential for high-level lysine production (M. Gubler et al., Appl. Microbiol. Biotechnol., 60, 857-863 (1994)).

Detail Description Paragraph - DETX (96):

[0114] **Carbon flux toward oxaloacetate may be increased** by overexpressing PEP carboxylase in conjunction with overexpressed pyruvate carboxylase without concoimtantly blocking carbon flux from PEP to pyruvate or affecting glucose uptake.

Detail Description Paragraph - DETX (97):

[0115] In heterotrophs such as C. glutamicum, however, PEP carboxylase requires acetyl-CoA for its activation, and is inhibited by aspartate (M. Jetten et al., Annals NY Acad. Sci., 272, 12-29 (1993)); hence amplification of C. glutamicum PEP carboxylase genes has not resulted in increased lysine yield (J. Cremer et al., Appl. Environ. Microbiol., 57, 1746-1752 (1991)). PEP carboxylase isolated from the cyanobacteria Anacystis nidulans, however, does not require acetyl CoA for activation nor is it inhibited by aspartate (M. Utter et al., Enzymes, 6, 117-135 (1972)). Therefore, this heterologous enzyme can be used to **increase the carbon flux** towards oxaloacetate in C. glutamicum. The genes encoding PEP carboxylase in A. nidulans have been isolated and cloned (T. Kodaki et al., J. Biochem., 97, 533-539 (1985)).

Detail Description Paragraph - DETX (124):

[0137] The one mole of **PEP formed in this reaction is available to PEP** carboxylase to generate OAA, or to pyruvate kinase to generate a second mole of pyruvate and ATP. The one mole committed to pyruvate is not available for direct conversion to OAA. Wild-type E. coli can still grow in the absence of the PTS, and a mutation in the glk gene for glucokinase is necessary to eliminate growth on glucose completely. Thus, a second route for glucose uptake involves glucose transport uncoupled from phosphorylation, a route which generally appears to be insignificant compared to the PTS in wild-type E. coli. The resulting net reaction may be expressed as:

Detail Description Paragraph - DETX (126):

[0138] In this case, two moles of **PEP are available to PEP** carboxylase for OAA formation. Of course, one mole of PEP could form pyruvate via pyruvate kinase with the generation of ATP so that the ultimate equations for the two routes to pyruvate are equivalent. In this study for anerobically grown cells, AFP111 showed markedly greater glucokinase activity than NZN111.

PGPUB-DOCUMENT-NUMBER: 20030068791

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030068791 A1

TITLE: Manufacture of five-carbon sugars and sugar alcohols

PUBLICATION-DATE: April 10, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 908744

DATE FILED: July 20, 2001

RELATED-US-APPL-DATA:

child 09908744 A1 20010720

parent continuation-in-part-of 09488581 20000121 US ABANDONED

child 09488581 20000121 US

parent continuation-in-part-of 08790585 19970129 US PENDING

child 08790585 19970129 US

parent continuation-of 08368395 19950103 US GRANTED

parent-patent 5631150 US

child 08368395 19950103 US

parent continuation-of 08110672 19930824 US ABANDONED

child 08110672 19930824 US

parent continuation-in-part-of 07973325 19921105 US ABANDONED

child 09908744 A1 20010720

parent continuation-in-part-of PCT/FI01/00051 20010122 US UNKNOWN

US-CL-CURRENT: 435/158, 435/252.3 , 435/254.2

ABSTRACT:

The invention relates to the methods of manufacturing five-carbon sugars and sugar alcohols as well as other compounds derived from pentose-phosphate pathway from readily available substrates such a hexoses using metabolically engineered microbial hosts.

---------- KWIC ---------

Detail Description Paragraph - DETX (11):

[0061] Xylulose-5-P can also be a precursor of several important products including xylulose, which in turn is a precursor for D-lyxose (via mannose-isomerase) and D-xylose (via xylose isomerase). Accordingly, the term "xylulose-5-P derived product" as used herein includes xylulose, D-lyxose and D-xylose, and mixtures of the same, but is not restricted to these examples. For example, a strain in which the genetic modifications results in increased relative amounts of xylulose-5-P can be further genetically modified to result in a strain with improved yields of xylulose-5-P derived products such as xylitol. Similarly, a strain that has been modified to have increased amounts of ribose-5-P or an **increased flux of carbon** to ribose-5-P can be further modified to increase the production of ribose-5-P derived products, and especially the production of nucleotides and riboflavin, or D-erythrose 4-P and products thereof, such as folate, ubiquinone and various aromatic amino acids. Similarly, as described herein for products such as sugar alcohols, production of ribose-5-P derived products in a strain accumulating ribose-5-P or having improved flux of carbon to ribose-5-P can be further improved by genetic modification of the subsequence/downstream metabolic reactions leading to such products.

Detail Description Paragraph - DETX (24):

[0074] For the enhanced production of ribulose-5-P, and ribulose-5-P-derived products, the disruption or inactivation of the ribose-5-P isomerase gene is especially preferred. Alternatively, or, in addition, the disruption or inactivation of the ribulose-5-P 3-epimerase gene is highly desirable. When such a host is cultivated on a six carbon sugar such as glucose, or a sugar that is converted into glucose or a six carbon sugar metabolite thereof such as glucose-6-P, carbon flow into the PPP is trapped or bottlenecked at the ribulose-5-P step, thus resulting in the accumulation of that intermediate, and in an **increased carbon flow** from ribulose-5-P into ribulose-5-P-derived products in those hosts that are capable of producing the same. By a production that is "trapped" or "bottlenecked" at a specific step, is meant

that the rate of utilization or degradation of the compound at that step by the host is less than the rate of synthesis of that compound, so that the amount of the compound is increased relative to hosts that do not contain this modification, when grown under the same conditions.

Detail Description Paragraph - DETX (25):

[0075] For the enhanced production of xylulose-5-P and xylulose-5-P-derived products, the disruption or inactivation of the gene encoding ribose-5-P isomerase is highly preferred. The gene encoding ribulose-5-P 3-epimerase is preferably either left intact or else additional copies (either homologous or heterologous but preferably homologous copies from the same species) of that gene are introduced, so as to enhance carbon flow into xylulose-5-P. Inactivation of the transketolase gene in addition is especially preferred when constructing a host for the enhanced capacity to produce xylulose-5-P. When such a host is cultivated on a six carbon sugar such as glucose, or a six carbon sugar metabolite thereof such as glucose-6-P, carbon flow into the PPP is trapped or bottlenecked at the xylulose-5-P step, thus resulting in the accumulation of that intermediate, and in an **increased carbon flow** from xylulose-5-P into xylulose-5-P-derived products in those hosts that are capable of producing the same.

Detail Description Paragraph - DETX (51):

[0101] In designing the hosts of the invention, it is of importance to also keep in mind the early steps of hexose metabolism, including the sugar uptake systems, the upper part of the glycolytic pathway (that is, at some point between hexokinase/glucokinase and aldolase action) and the oxidative branch of the PPP. Genetic modifications in those areas are not required for the implementation of this invention. However, the hosts of the invention can be genetically modified to contain one or more **modifications in such areas so as to maximizing the carbon flow** into the oxidative branch of the PPP and thus into the non-oxidative branch of the PPP, thus resulting in a further improvement in the yields of the desired fermentation products.

Detail Description Paragraph - DETX (54):

[0104] An alternative/complementary way of achieving **increased carbon flow** into the PPP is the over-expression of a gene coding the first enzyme of the oxidative branch of PPP: glucose 6-phosphate dehydrogenase. Particularly, such genes from heterofermentative lactic acid bacteria (e.g., Leuconostoc mesenteroides) or Zymomonas mobilis (GenBank accession number M60615) would be suitable because of their reduced sensitivity towards allosteric inhibition typical of many glucose 6-phosphate dehydrogenases (Sugimoto S. & Shio, I., Agric. Biol. Chem. 51:101-108 (1987)). Over-expression of a gene coding for the second enzyme of the oxidative branch of PPP, 6-phosphogluconate, is also considered to be within the scope of current invention. High activity of this enzyme can prevent the cells from accumulating 6-phosphogluconate and excreting gluconic acid into the culture medium.

Detail Description Paragraph - DETX (59):

[0109] A modification of the glucose uptake system is advantageous when this

invention is implemented in a bacterial host that takes up and phosphorylates glucose via the PTS system. The functioning of the PTS system requires a continuous **supply of phosphoenolpyruvate**--a product of the glycolytic pathway. If the PTS system is replaced with a glucokinase- or hexokinase-based glucose uptake and phosphorylation system, then ATP rather than **phosphoenolpyruvate would supply** the energy for glucose uptake and phosphorylation. Unlike phosphoenolpyruvate, ATP can be replenished via the respiratory chain, utilizing NADPH generated by the oxidative branch of PPP. Thus, such a system would provide a much better energy balance for those microbial host cells of the invention that convert hexose-phosphates into pentose phosphates via the PPP and consequently higher yields of the desired five-carbon sugars would result. The technology for replacement of a PTS-based glucose uptake and phosphorylation system with a kinase-based system is known in the art (Flores, N., et al., Nature Biotechnology 14:620-623 (1996)). The invention is also directed to a modified glucose uptake mechanism, which results in the enhanced flow of glucose and intermediates derived from glucose into the pentose phosphate, by over expression of the B. subtilis glcUgdh operon. Such hosts are especially useful when it is desired to utilize a host that produces an enhanced level of one or more pentose phophate shunt intermediates, for example, in the methods of making xylitol as described herein. The invention is also directed to a host, which has been genetically modified to enhance the expression of the glcUgdh operon.

Detail Description Paragraph - DETX (236):

Enhancement of Glucose **Carbon Flow into the Pentose-phosphate Pathway and Modification** of Glucose Uptake System in Bacillus subtilis

Claims Text - CLTX (56):

55. The method of any one of claims 1, 17, 32 or 44, wherein said host has an **increased carbon flux** into the oxidative branch of the pentose phosphate pathway during said cultivating of part (A) when compared to said host prior to said genetic modification.

Claims Text - CLTX (96):

95. The host of any one of claims 90, 91, 92 or 93, wherein said host has an **increased carbon flux** into the oxidative branch of the pentose phosphate pathway when compared to said host prior to said genetic modification.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	4270	carbon near2 (flux or flow)	USPAT; US-PGPUB	2003/12/24 07:56
2	L2	275	1 near4 (modif$8 or alter$8 or increas$8)	USPAT; US-PGPUB	2003/12/24 07:56
3	L3	166	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT; US-PGPUB	2003/12/24 07:57
4	L4	9	2 and 3	USPAT; US-PGPUB	2003/12/24 07:57
5	L5	6159	phosphotransferase$1 or phospho adj transferase$1	USPAT; US-PGPUB	2003/12/24 08:10
6	L6	36	(2 or 3) and 5	USPAT; US-PGPUB	2003/12/24 08:11

PGPUB-DOCUMENT-NUMBER: 20030233670

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030233670 A1

TITLE: Gene sequences and uses thereof in plants

PUBLICATION-DATE: December 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Chomet, Paul S.	Mystic	CT	US	
Laccetti, Lucille B.	Groton	CT	US	

APPL-NO: 10/ 310154

DATE FILED: December 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60337358 20011204 US

US-CL-CURRENT: 800/278, 435/200 , 435/320.1 , 435/419 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

The invention provides polynucleotides and proteins encoded by the polypeptides. The disclosed polynucleotides and polypeptides find use in production of transgenic plants to produce plants having improved properties. The invention further provides methods of producing fertile transgenic plants, preferably maize, with desirable phenotypes and progeny of any generation derived from the fertile transgenic plants.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn.119(e) of U.S. Provisional Application No. 60/337,358 filed Dec. 4, 2001, the disclosure of which application is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (129):

[0156] Cytokinins promote and sustain plant cell division (Riou-Khamlichi et al., 1999), modulate developmental processes such as apical dominance (Napoli et al., 1999) and senescence (Gan and Amasino, 1995), play a role in nitrogen

(Yu et al, 1998) and carbon (Miyazawa et al., 1999) metabolism and interact with most other phytohormone signaling pathways (Cary et al., 1995; Kusnetsov et al., 1998; Miyazawa et al., 1999). Manipulation of cytokinin levels or signaling pathways can thus be expected to improve yield by increasing kernel cell numbers, delaying senescence, increasing the number of ears a corn plant sets, antagonizing abscisic acid signaling, improving nitrogen use efficiency or **increasing carbon flow** into amyloplasts. However, the diverse roles that cytokinins play in plant growth and development also suggest that temporal and spatial control will be required to manipulate cytokinin signaling for plant improvement.

Detail Description Paragraph - DETX (583):

[0610] It is believed that DNA is introduced into only a small percentage of target cells in any one experiment. In order to provide an efficient system for identification of those cells receiving DNA and integrating it into their genomes one may employ a means for selecting those cells that are stably transformed. One exemplary embodiment of such a method is to introduce into the host cell, a marker gene which confers resistance to some normally inhibitory agent, such as an antibiotic or herbicide. Examples of antibiotics which may be used include the aminoglycoside antibiotics neomycin, kanamycin, G418 and paromomycin, or the antibiotic hygromycin. Resistance to the aminoglycoside antibiotics is conferred by aminoglycoside phosphostransferase enzymes such as neomycin **phosphotransferase** II (NPT II) or NPT I, whereas resistance to hygromycin is conferred by hygromycin **phosphotransferase**.

Detail Description Paragraph - DETX (675):

[0700] A GATEWAY.TM. Destination (Invitrogen Life Technologies, Carlsbad, Calif.) plant expression vector was constructed (pMON65154, FIG. 1) using methods known to those of skill in the art. The elements of the expression vector are summarized in 7. The backbone of the plasmid pMON65154 comprising the bacterial replication functions and an ampicillin resistance gene expressed in E. coli were derived from the plasmid pSK-. The plant expression elements in pMON64154 are available to those of skill in the art and references are provided for each element in Table 2. All references in Table 2 to location refer to base pair coordinates for each element on the plasmid-map disclosed in FIG. 1. Generally, pMON65 154 comprises a selectable marker expression cassettte comprising a Cauliflower Mosaic Virus 35S promoter operablly linked to a gene encoding neomycin **phosphotransferase** II (nptII). The 3' region of the selectable marker expression cassette comprises the 3' region of the Agrobacterium tumefaciense nopaline synthase gene (nos) followed 3' by the 3' region of the potato proteinase inhibitor II (pinII) gene. The plasmid pMON 65154 further comprises a plant expression cassette into which a gene of interest may be inserted using GATEWAY.TM. cloning methods. The GATEWAY.TM. cloning cassette is flanked 5' by a rice actin 1 promoter, exon and intron and flanked 3' by the 3' region of the potato pinII gene. Using GATEWAY.TM. methods, the cloning cassette was replaced by a gene of interest. The vector pMON65154 and derivaties thereof comprising a gene of interest, were particularly useful in methods of plant transformation via direct DNA delivery, such as microprjectile bombardment. One of skill in the art could construct an expression vector with similar features using methods known in the art. Furthermore, one of skill in the art would appreciate that other promoters and

3' regions would be useful for expression of a gene of interest and other selectable markers may be used.

Detail Description Paragraph - DETX (723):

[0741] Transformants were selected on culture medium comprising paromomycin, based on expression of a transgenic neomycin **phosphotransferase** II (nptII) gene. Twenty four hours after DNA delivery, tissue was transferred to 211V medium containing 25 mg/L paromomycin (medium 211HV). After three weeks incubation in the dark at 27.degree. C., tissue was transferred to medium 211 containing 50 mg/L paromomycin (medium 211G). Tissue was transferred to medium 211 containing 75 mg/L paromomycin (medium 211XX) after three weeks. Transformants were isolated following 9 weeks of selection. Table 4 (Example 9) dislcoses results of transformant experiments using the methods of microprojectile bombardment disclosed herein.

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TITLE: Regulating metabolism by modifying the level of trehalose-6-phosphate by inhibiting endogenous trehalase levels

PUBLICATION-DATE: September 18, 2003

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WO	PCT/EP97/02497	1997WO-PCT/EP97/02497	May 2, 1997

US-CL-CURRENT: 800/284, 800/317.2

ABSTRACT:

A method of modification of the development and/or composition of cells, tissues, or organs in vivo in plants by inhibiting the level of an endogenous trehalase is provided. The cells, tissues, or organs have been genetically altered to comprise a DNA sequence encoding a trehalase inhibitor. The DNA sequence is capable of expressing an RNA that is at least partially complementary to an RNA produced by a DNA sequence encoding the endogenous trehalase. Alternatively, the DNA sequence comprises a DNA sequence which is identical to a DNA sequence encoding the endogenous trehalase. The modification is other than to increase production or accumulation of trehalose.

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Brief Description of Drawings Paragraph - DRTX (2):

[0020] FIG. 1. Schematic representation of plasmid pVDH275 harbouring the neomycin-**phosphotransferase** gene (NPTII) flanked by the 35S cauliflower mosaic virus promoter (P35S) and terminator (T35S) as a selectable marker; an expression cassette comprising the pea plastocyanin promoter (pPCpea) and the nopaline synthase terminator (Tnos); right (RB) and left (LB) T-DNA border sequences and a bacterial kanamycin resistance (KanR) marker gene.

Detail Description Paragraph - DETX (17):

[0043] In plants, generation of the "plenty" signal by decreasing the intracellular concentration of trehalose-6-phosphate through expression of the enzyme TPP (or inhibition of the enzyme TPS) will signal all cell systems to **increase glycolytic carbon flow** and inhibit photosynthesis. This is nicely shown in WO 97/42326, where for instance in Experiment 2 transgenic tobacco plants are described in which the enzyme TPP is expressed having increased leaf size, increased branching and a reduction of the amount of chlorophyll. However, since the "plenty" signal is generated in the absence of sufficient supply of glucose, the pool of carbohydrates in the cell is rapidly depleted.

Detail Description Paragraph - DETX (40):

[0066] To select or screen for transformed cells, it is preferred to include a marker gene linked to the plant expressible gene according to the invention to be transferred to a plant cell. The choice of a suitable marker gene in plant transformation is well within the scope of the average skilled worker; some examples of routinely used marker genes are the neomycin **phosphotransferase** genes conferring resistance to kanamycin (EP-B 131 623), the glutathion-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides (EP-A 256 223), glutamine synthetase conferring upon overexpression resistance to glutamine synthetase inhibitors such as phosphinothricin (WO 87/05327), the acetyl transferase gene from Streptomyces viridochromogenes conferring resistance to the selective agent phosphinothricin (EP-A 275 957), the gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine, the bar gene conferring resistance against Bialaphos (e.g. WO 91/02071), the cah gene conferring resistance to cyanamide and the like. The actual choice of the marker is not crucial as long as it is functional (i.e. selective) in combination with the plant cells of choice.

Claims Text - CLTX (5):

4. The method according to claim 1, wherein said **modification comprises inhibition of carbon flow** in the glycolytic direction.

Claims Text - CLTX (14):

13. The method according to claim 10, wherein said **modification comprises**

inhibition of carbon flow in the glycolytic direction.

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TITLE: Transgenic plants containing altered levels of steroid compounds

PUBLICATION-DATE: August 7, 2003

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US-CL-CURRENT: 800/278, 435/189 , 435/410 , 536/23.2

ABSTRACT:

Disclosed are constructs comprising sequences encoding 3-hydroxy-3methylglutaryl-Coenzyme A reductase and at least one other sterol synthesis pathway enzyme. Also disclosed are methods for using such constructs to alter sterol production and content in cells, plants, seeds and storage organs of plants. Also provided are oils and compositions containing altered sterol levels produced by use of the disclosed constructs. Novel nucleotide sequences useful in the alteration of sterol production are also provided. Also provided are cells, plants, seeds and storage organs of plants comprising sequences encoding 3-hydroxy-3methylglutaryl-Coenzyme A reductase, at least one other sterol synthesis pathway enzyme and at least one tocopherol synthesis enzyme.

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Brief Description of Drawings Paragraph - DRTX (8):

[0052] FIG. 6 is a map showing the structure of construct pMON43818. pMON43818 is a recombinant binary vector carrying the gene encoding rubber

hydroxymethyl glutaryl CoA reductasel (HMGR1) in sense orientation driven by the soybean alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin **phospho transferase** protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Rubber HMGR1 gene: coding sequence for HMGR1 gene from Hevea brasiliensis; E9 3': 3' end of pea rbcS E9 gene; Left border: octopine left border, sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; Ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Brief Description of Drawings Paragraph - DRTX (9):

[0053] FIG. 7 is a map showing the structure of construct pMON43052. pMON43052 is a recombinant shuttle vector, carrying the cDNA fragment encoding the catalytic domain of Arabidopsis HMGR1 in sense orientation driven by the soybean alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin **phosphotransferase** protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Arabidopsis HMGR catalytic domain: coding sequence for the catalytic domain of Arabidopsis HMGR1 protein; E9 3': 3' end of pea rbcS E9 gene; Left border: octopine left border, sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; Ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Brief Description of Drawings Paragraph - DRTX (10):

[0054] FIG. 8 is a map showing the structure of construct pMON51850. pMON51850 is a binary vector for Agrobacterium mediated transformation of soybean. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin **phosphotransferase** protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-v: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Brief Description of Drawings Paragraph - DRTX (11):

[0055] FIG. 9 is a map showing the structure of construct pMON43057. pMON43057 is a recombinant binary vector for Agrobacterium mediated transformation of soybean, carrying the gene cassette for expressing catalytic domain of HMGR1 from Arabidopsis thaliana. The catalytic domain of the HMGRI

CDNA is driven by soybean 7S alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin **phosphotransferase** protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence essential for transfer of T-DNA into Agrobacterium; Soy Alpha' Beta Conglycinin: soybean 7S alpha' beta conglycinin gene promoter; Arabidopsis HMGR catalytic domain: coding sequence for Arabidopsis HMGR1 catalytic domain; E9 3': 3' end of pea rbcS E9 gene.

Brief Description of Drawings Paragraph - DRTX (12):

[0056] FIG. 10 is a map showing the structure of construct pMON43058. pMON43058 is a recombinant binary vector for Agrobacterium-mediated soybean transformation, carrying gene expression cassettes for catalytic domain of HMGR1 from Arabidopsis thaliana and SMTII from Arabidopsis thaliana. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin **phosphotransferase** protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence essential for transfer of T-DNA into Agrobacterium; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Arabidopsis HMGR catalytic domain: sequence encoding the catalytic domain of Arabidopsis HMGR1; E9 3': 3' end of pea rbcS E9 gene; Soy Alpha' Beta Conglycinin: soybean 7S alpha' beta conglycinin gene promoter; Arabidopsis SMT2: cDNA encoding sterol methyl transferase II enzyme from Arabidopsis thaliana (accession no: X89867); NOS 3': 3' termination end of nopaline synthase coding region.

Detail Description Paragraph - DETX (206):

[0283] In preferred embodiments, the vector used to express the polypeptide coding gene includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin resistance; i.e., the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin **phosphotransferase** II and nopaline synthase 3' nontranslated region described by Rogers et al., in Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach eds., Academic Press Inc., San Diego, Calif. (1988).

Detail Description Paragraph - DETX (341):

[0416] Table 2: Sterol profile of transgenic soybean ants expressing rubber HMGR gene driven by 7s omoter. Event 1:control, events 2-16:15 transgenic ants generated by 15 independent events using robacterium mediated transformation. Total sterols increased by 3.2- and 3.9- fold in e best performing plants (transgenic events 3 and 4). ese two events also showed the highest increases

of dividual sterols. Campesterol increased by 2.7-fold, tosterol by 3.4-fold, sitostanol by 3.2-fold and her sterols by 6.5-fold in event 3 while stigmasterol creased by 2.3-fold in event 4. The other sterols, ich account for the highest increase in total sterols re pathway intermediates that included squalene, cloartenol, 24-methylene cycloartenol, obtusifoliol, ofucosterol, and stigmasta-7-enol. These pathway ntermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to **increased carbon flux** through the pathway, they accumulate in significant amounts. This suggests additional control points for sterol biosynthesis in plants such as squalene epoxidase, C-24 sterol methyltransferase, and C-14 obtusifoliol demethylase.

Detail Description Paragraph - DETX (353):

[0427] Six transgenic lines haboring pMON43058 produced 5.8- to 6-fold increase in total sterols and the rest of the 10 transgenic lines with the pMON43058 showed 3- to 5-fold increase in total sterols. The best performing transgenic lines showed about 2- to 3-fold increase in sitosterol and 4.5- to 6-fold increase in sitostanol levels. However, the campesterol accumulation was reduced by 50% in these lines. This was due to overexpression of the Arabidopsis SMTII enzyme which enhances the carbon flux towards the synthesis of 24-ethyl sterols thereby reducing the carbon flux through the pathway leading to the synthesis of 24-methyl sterols. As seen in pMON43057 transgenic lines, all of the transgenic lines harboring the pMON43058 also accumulated 50-60% of the total sterols in the form of pathway intermediates which are squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, isofucosterol, and stigmasta-7-enol. These pathway intermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to **increased carbon flux** through the pathway, they accumulate in significant amounts. The pathway intermediates accumulation is highly significant when the truncated from of HMGR is overexpressed as compared to the full length form of HMGR suggesting that the overexpression of the truncated form of HMGR creates even greater **increase in carbon flux** through the pathway. This provides further evidence for additional control points for sterol biosynthesis in plants such as squalene epoxidase, sterol methyltransferase I, sterol C4-demethylase, obtusifoliol C14.alpha.-demethylase, sterol C5-desaturase, and sterol methyl transferase II.

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TITLE: Microorganisms and methods for overproduction of DAHP by cloned Pps gene

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INVENTOR-INFORMATION:

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ABSTRACT:

Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

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Summary of Invention Paragraph - BSTX (15):

[0014] The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common aromatic pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

Summary of Invention Paragraph - BSTX (19):

[0017] The present invention further provides a method for **increasing carbon**

flow for the biosynthesis of DAHP in a host cell comprising the steps of transforming into the host cell recombinant DNA comprising a pps gene so that Pps is expressed at enhanced levels relative to wild type host cells, concentrating the transformed cells through centrifugation, resuspending the cells in a minimal, nutrient lean medium, fermenting the resuspended cells, and isolating DAHP from the medium.

Summary of Invention Paragraph - BSTX (20):

[0018] The present invention further provides methods of **increasing carbon flow** into the common aromatic pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

Detail Description Paragraph - DETX (3):

[0031] The inventor have found that cell lines can be developed that **increase the carbon flux** into DAHP production and achieve near theoretical yields of DAHP by overexpressing phosphoenolpyruvate synthase (Pps) in the cell lines. Overexpression of Pps can increase the final concentration and yield of DAHP by as much as two-fold, to a near theoretical maximum as compared to wild type cell lines. The overexpression of Pps is achieved by transforming a cell line with recombinant DNA comprising a pps gene so that Pps is expressed at enhanced level relative to the wild type cell line and so that the yield of DAHP approaches its theoretical value.

Detail Description Paragraph - DETX (5):

[0033] Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common aromatic pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-aromatic, and aromatic metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common aromatic pathway relative to wild type host cells not containing the genetic elements of the present invention.

Detail Description Paragraph - DETX (6):

[0034] In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common aromatic pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common aromatic pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps gene so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium where the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

Detail Description Paragraph - DETX (8):

[0036] Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common aromatic pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

Detail Description Paragraph - DETX (12):

[0040] Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the **phosphotransferase** system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common aromatic pathway or pathways branching therefrom.

Detail Description Paragraph - DETX (15):

[0043] A key component of the methods of the present invention directed at **increased carbon flux** commitment to DAHP and DAHP metabolites is the recycling of pyruvate to PEP. Pyruvate is available in host cells as an end product of glycolysis.

Detail Description Paragraph - DETX (47):

[0074] As shown in FIG. 3A, for maximum yield of DAHP production by strains without Pps overproduction, 7 moles of glucose are needed to produce 3 moles of DAHP (43% molar yield) and 7 moles of pyruvate which is further metabolized. The relative flux through each intermediate step is also shown in FIG. 3A. The formation of pyruvate is necessary because of the stoichiometry of the **phosphotransferase** system for glucose uptake.

Detail Description Paragraph - DETX (52):

[0079] The stimulation of glucose consumption in the previous work was attributed to the altered PEP/pyruvate ratio. It was hypothesized that increased PEP/pyruvate ratio stimulates the **phosphotransferase** system for increased glucose consumption, which in turn results in the excretion of pyruvate.

Detail Description Paragraph - DETX (93):

[0119] This example demonstrates that the E. Coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the aromatic pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of

transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pATI (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

Detail Description Paragraph - DETX (96):

[0122] To determine whether the Pps effect requires overexpressed transketolase (Tkt) as well, plasmid pRW5, which contains only aroG.sup.fbr, was used in place of pATI in the above experiments. It was found that overproduction of Pps did not increase the DAHP production (FIG. 2A) without the elevated Tkt activity. Therefore, as limitation of small molecules in the biosynthesis of DAHP is concerned, the first limitation arises from the supply of E4P. This bottleneck shifts to the **supply of PEP** when Tkt is overexpressed, which is believed to increase the supply of E4P.

Detail Description Paragraph - DETX (99):

[0124] Since PEP is also converted to OAA by Ppc, the deletion of this enzyme may increase the **supply of PEP**. Therefore, the ppc gene on the chromosome of AB2847 was inactivated to determine whether DAHP production could be increased without Pps overexpression. This was done by transducing AB2847 with a PI lysate grown on JCL1242 ppc::Km. The resulting transductant, JCL1283 aroB ppc::Km was then transformed with pATI or pRW5 and tested for DAHP production in the re-suspension culture as described above. To avoid limitation of OAA in the ppc strain, the culture medium was supplemented with succinate, which was shown to have no effect on DAHP production (data not shown). Contrary to the expectation, ppc mutation did not increase the production of DAHP (FIG. 2B), suggesting that the metabolic flux from PEP to OAA was not significant under the experimental conditions tested here. In fact, the ppc mutation actually decreased the DAHP production for unknown reasons.

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TITLE: Expression of fructose 1,6 bisphosphate aldolase in transgenic plants

PUBLICATION-DATE: July 3, 2003

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ABSTRACT:

Fructose-1,6-bisphosphate aldolase (FDA) is an enzyme reversibly catalyzing the reaction converting triosephosphate into fructose-1,6-bisphosphate. In the leaf, this enzyme is located in the chloroplast (starch synthesis) and the cytosol (sucrose biosynthesis). Transgenic plants were generated that express the E. coli fda gene in the chloroplast to improve plant yield by increasing leaf starch biosynthetic ability in particular and sucrose production in general. Leaves from plants expressing the fda transgene showed a significantly higher starch accumulation, as compared to control plants expressing the null vector, particularly early in the photoperiod, but had lower leaf sucrose. Transgenic plants also had a significantly higher root mass. Furthermore, transgenic potatoes expressing fda exhibited improved uniformity of solids.

[0001] This application is based on U.S. Provisional Application Serial No. 60/049,995, filed Jun. 17, 1997.

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Summary of Invention Paragraph - BSTX (26):

[0024] First, increasing the expression of the FDA enzyme in the chloroplast would **increase the flow of carbon through the Calvin Cycle and increase** atmospheric carbon assimilation during early photoperiod. This would result in an increase in photosynthetic efficiency and an increase in chloroplast starch production (a leaf carbon storage form degraded during periods when photosynthesis is low or absent). Both of these responses would lead to an increase in sucrose production by the leaf and a net increase in carbon export during a given photoperiod. This increase in source capacity is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Summary of Invention Paragraph - BSTX (28):

[0026] Third, expression of FDA in sink tissues can show several desirable traits, such as increased amino acid and/or fatty acid pools via **increases in carbon flux** through glycolysis (and thus pyruvate levels) in seeds or other sinks and increased starch levels as result of increased production of glucose 6-phosphate in seeds, roots, stems, and tubers where starch is a major storage nonstructural carbohydrate (reverse glycolysis). This increase in sink strength is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Detail Description Paragraph - DETX (2):

[0038] This invention is directed to a method for producing plant cells and plants demonstrating an increased or improved growth and development, yield, quality, starch storage uniformity, vigor, and/or stress tolerance. The method utilizes a DNA sequence encoding an fda (fructose 1,6 bisphosphate aldolase) gene integrated in the cellular genome of a plant as the result of genetic engineering and causes expression of the FDA enzyme in the transgenic plant so produced. Plants that overexpress the FDA enzyme exhibit **increased carbon flow through the Calvin Cycle and increased** atmospheric carbon assimilation during early photoperiod resulting in an increase in photosynthetic efficiency and an increase in starch production. Thus, such plants exhibit higher levels of sucrose production by the leaf and the ability to achieve a net increase in carbon export during a given photoperiod. This increase in source capacity leads to increased plant growth that in turn generates greater biomass and/or increases the size of the sink and ultimately providing greater yields of the transgenic plant. This greater biomass or increased sink size may be evidenced in different ways or plant parts depending on the particular plant species or growing conditions of the plant overexpressing the FDA enzyme. Thus, increased size resulting from overexpression of FDA may be seen in the seed, fruit, stem, leaf, tuber, bulb or other plant part depending upon the plant species and its dominant sink during a particular growth phase and upon the environmental effects caused by certain growing conditions, e.g. drought, temperature or other stresses. Transgenic plants overexpressing FDA may therefore have increased carbon assimilation, export and storage in plant source and sink

organs, which results in growth, yield, and uniformity and quality improvements.

Detail Description Paragraph - DETX (3):

[0039] Plants overexpressing FDA may also exhibit desirable quality traits such as increased production of starch, oils and/or proteins depending upon the plant species overexpressing the FDA. Thus, overexpression of FDA in a particular plant species may affect or **alter the direction of the carbon flux** thereby directing metabolite utilization and storage either to starch production, protein production or oil production via the role of FDA in the glycolysis and gluconeogenesis metabolic pathways.

Detail Description Paragraph - DETX (36):

[0072] A recombinant DNA molecule of the invention typically includes a selectable marker so is that transformed cells can be easily identified and selected from non-transformed cells. Examples of such include, but are not limited to, a neomycin **phosphotransferase** (nptII) gene (Potrykus et al., 1985), which confers kanamycin resistance. Cells expressing the nptII gene can be selected using an appropriate antibiotic such as kanamycin or G418. Other commonly used selectable markers include the bar gene, which confers bialaphos resistance; a mutant EPSP synthase gene (Hinchee et al., 1988), which confers glyphosate resistance; a nitrilase gene, which confers resistance to bromoxynil (Stalker et al., 1988); a mutant acetolactate synthase gene (ALS), which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204, 1985); and a methotrexate resistant DHFR gene (Thillet et al., 1988).

Detail Description Paragraph - DETX (87):

[0118] This increase in sucrose export by fda-expressing leaves is an illustration of an increase in source capacity, very likely due to an **increased carbon flow** through the Calvin Cycle (in response to increased triose-P utilization) and thus an increase in net carbon utilization by the leaf. As seen in Table 2, the increase in sucrose loading in the phloem correlates with the level of fda expression.

Detail Description Paragraph - DETX (94):

[0123] For the cytosolic expression of the fda gene in corn plants, a construct was made in which the fda gene sequence was fused to the backbone of a vector containing the enhanced CaMV 35S promoter (e35S; Kay et al., 1987), the HSP70 intron (U.S. Pat. No. 5,593,874), and the NOS3' polyadenylation sequence (Fraley et al., 1983). This created a NotI cassette [P-e35S/HSP70 intron/fda/NOS3'] that was cloned into the NotI site of pMON30460, a monocot transformation vector, to form the plant transformation vector pMON13925, as shown in FIG. 5. pMON30460 contains an expression cassette for the selectable marker neomycin **phosphotransferase** typeII gene (nptII) [P-35S/NPTII/NOS3'] and a unique NotI site for cloning the gene of interest. The final vector (pMON13925) was constructed so that the gene of interest and the selectable marker gene were cloned in the same orientation. A vector fragment containing the expression cassettes for these gene sequences could be excised from the bacterial selector (Kan) and ori, gel purified, and used for plant

transformation.

Detail Description Paragraph - DETX (98):

[0127] Transgenic maize plants transformed with the vectors pMON13925 (described above) or pMON17590 (described above) were produced using microprojectile bombardment, a procedure well-known to the art (Fromm, 1990; Gordon-Kamm et al., 1990; Walters et al., 1992). Embryogenic callus initiated from immature maize embryos was used as a target tissue. Plasmid DNA at 1 mg/mL in TE buffer was precipitated onto M10 tungsten particles using a calcium chloride/spermidine procedure, essentially as described by Klein et al. (1988). In addition to the gene of interest, the plasmids also contained the neomycin **phosphotransferase** II gene (nptII) driven by the 35S promoter from Cauliflower Mosaic Virus. The embryogenic callus target tissue was pretreated on culture medium osmotically buffered with 0.2M mannitol plus 0.2M sorbitol for approximately four hours prior to bombardment (Vain et al., 1993). Tissue was bombarded two times with the DNA-coated tungsten particles using the gunpowder version of the BioRad Particle Delivery System (PDS) 1000 device. Approximately 16 hours following bombardment, the tissue was subcultured onto a medium of the same composition except that it contained no mannitol or sorbitol, and it contained an appropriate aminoglycoside antibiotic, such as G418", to select for those cells that contained and expressed the 35S/nptII gene. Actively growing tissue sectors were transferred to fresh selective medium approximately every 3 weeks. About 3 months after bombardment, plants were regenerated from surviving embryogenic callus essentially as described by Duncan and Widholm (1988).

Detail Description Paragraph - DETX (113):

[0140] A second potato transformation vector was constructed by cloning the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the unique NotI site of pMON23616. pMON23616 is a potato transformation vector containing the nopaline-type T-DNA right border region (Fraley et al., 1985), an expression cassette for the neomycin **phosphotransferase** typeII gene [P-35S/NPTII/NOS3'] (selectable marker), a unique NotI site for cloning the gene expression cassette of interest, and the T-DNA left border region (Barker et al., 1983). Cloning of the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the NotI site of pMON23616 results in the potato transformation vector pMON17581, as shown in FIG. 8. The vector pMON17581 was constructed such that the gene of interest and the selectable marker gene were transcribed in the same direction.

PGPUB-DOCUMENT-NUMBER: 20030087381

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087381 A1

TITLE: Metabolically engineered organisms for enhanced production of oxaloacetate-derived biochemicals

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

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Eiteman, Mark A.	Athens	GA	US	
Altman, Elliot	Athens	GA	US	

APPL-NO: 10/ 215440

DATE FILED: August 9, 2002

RELATED-US-APPL-DATA:

child 10215440 A1 20020809

parent continuation-in-part-of 09417557 19991013 US GRANTED

parent-patent 6455284 US

child 09417557 19991013 US

parent continuation-in-part-of PCT/US99/08014 19990413 US PENDING

non-provisional-of-provisional 60081598 19980413 US

non-provisional-of-provisional 60082850 19980423 US

US-CL-CURRENT: 435/69.1, 435/193 , 435/252.3 , 435/252.33 , 435/320.1 , 536/23.2

ABSTRACT:

Metabolic engineering is used to **increase the carbon flow** toward oxaloacetate to enhance production of bulk biochemicals, such as lysine and succinate, in industrial fermentations. Carbon flow is redirected by genetically engineering the cells to overexpress the enzyme pyruvate carboxylase.

[0001] This application is a continuation-in-part application of U.S. application Ser. No. 09/417,557, filed Oct. 13, 1999, which is a continuation-in-part of International Application PCT/US99/08014, with an international filing date of Apr. 13, 1999, which in turn claims the benefit

of U.S. Provisional Application No. 60/081,598, filed Apr. 13, 1998, and U.S. Provisional Application No. 60/082,850, filed Apr. 23, 1998, each of which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Metabolic engineering is used to **increase the carbon flow** toward oxaloacetate to enhance production of bulk biochemicals, such as lysine and succinate, in industrial fermentations. Carbon flow is redirected by genetically engineering the cells to overexpress the enzyme pyruvate carboxylase.

Summary of Invention Paragraph - BSTX (8):

[0008] PEP occupies a central position, or node, in carbohydrate metabolism. As the final intermediate in glycolysis, and hence the immediate precursor in the formation of pyruvate via the action of the enzyme pyruvate kinase, it can serve as a source of energy. Additionally, PEP can replenish intermediates in the TCA cycle via the anaplerotic action of the enzyme PEP carboxylase, which converts PEP directly into the TCA intermediate oxaloacetate. PEP is also often a cosubstrate for glucose uptake into the cell via the **phosphotransferase** system (PTS) and is used to biosynthesize aromatic amino acids. In many organisms, TCA cycle intermediates can be regenerated directly from pyruvate. For example, pyruvate carboxylase (PYC), which is found in some bacteria but not E. coli or Salmonella typhimurium, mediates the formation of oxaloacetate by the carboxylation of pyruvate utilizing carboxybiotin. As might be expected, the partitioning of PEP is rigidly regulated by cellular control mechanisms, causing a metabolic "bottleneck" which limits the amount and direction of carbon flowing through this juncture. The enzyme-mediated conversions that occur between PEP, pyruvate and oxaloacetate are shown in FIG. 3.

Summary of Invention Paragraph - BSTX (10):

[0010] Various metabolic engineering strategies have been pursued, with little success, in an effort to overcome the network rigidity that surrounds carbon metabolism. For example, overexpression of the native enzyme PEP carboxylase in E. coli was shown to **increase the carbon flux** towards oxaloacetate (C. Millard et al., Appl. Environ. Microbiol., 62, 1808-1810 (1996); W. Farmer et al, Appl. Env. Microbiol., 63, 3205-3210 (1997)); however, such genetic manipulations also cause a decrease in glucose uptake (P. Chao et al., Appl. Env. Microbiol., 59, 4261-4265 (1993)), since PEP is a required cosubstrate for glucose transport via the **phosphotransferase** system. An attempt to improve lysine biosynthesis in Corynebacterium glutamicum by overexpressing PEP carboxylase was likewise not successful (J. Cremer et al., Appl. Env. Microbiol., 57, 1746-1752 (1991)). In another approach to divert carbon flow toward oxaloacetate, the glyoxylate shunt in E. coli was derepressed by knocking out one of the transcriptional regulators, fadR. Only a slight increase in biochemicals derived from oxaloacetate was observed (W. Farmer et al., Appl. Environ. Microbiol., 63, 3205-3210 (1997)). In a

different approach, malic enzyme from Ascaris suum was overproduced in mutant E. coli which were deficient for the enzymes that convert pyruvate to lactate, acetyl-CoA, and formate. This caused pyruvate to be converted to malate which increased succinate production (see FIG. 2). However, this approach is problematic, since the mutant strain in question cannot grow under the strict anaerobic conditions which are required for the optimal fermentation of glucose to organic acids (L. Stols et al., Appl. Biochem. Biotechnol., 63-65, 153-158 (1997)).

Detail Description Paragraph - DETX (95):

[0113] In many organisms PEP can be carboxylated to oxaloacetate via PEP carboxylase or it can be converted to pyruvate by pyruvate kinase (I. Shiio et al., J. Biochem., 48, 110-120 (1960);M. Jetten et al., Appl. Microbiol. Biotechnol., 41, 47-52 (1994)). One possible strategy that was tried to **increase the carbon flux** toward oxaloacetate in C. glutamicum was to block the carbon flux from PEP toward pyruvate (see FIG. 3). However, lysine production by pyruvate kinase mutants was 40% lower than by a parent strain, indicating that pyruvate is essential for high-level lysine production (M. Gubler et al., Appl. Microbiol. Biotechnol., 60, 857-863 (1994)).

Detail Description Paragraph - DETX (96):

[0114] **Carbon flux toward oxaloacetate may be increased** by overexpressing PEP carboxylase in conjunction with overexpressed pyruvate carboxylase without concoimtantly blocking carbon flux from PEP to pyruvate or affecting glucose uptake.

Detail Description Paragraph - DETX (97):

[0115] In heterotrophs such as C. glutamicum, however, PEP carboxylase requires acetyl-CoA for its activation, and is inhibited by aspartate (M. Jetten et al., Annals NY Acad. Sci., 272, 12-29 (1993)); hence amplification of C. glutamicum PEP carboxylase genes has not resulted in increased lysine yield (J. Cremer et al., Appl. Environ. Microbiol., 57, 1746-1752 (1991)). PEP carboxylase isolated from the cyanobacteria Anacystis nidulans, however, does not require acetyl CoA for activation nor is it inhibited by aspartate (M. Utter et al., Enzymes, 6, 117-135 (1972)). Therefore, this heterologous enzyme can be used to **increase the carbon flux** towards oxaloacetate in C. glutamicum. The genes encoding PEP carboxylase in A. nidulans have been isolated and cloned (T. Kodaki et al., J. Biochem., 97, 533-539 (1985)).

Detail Description Paragraph - DETX (122):

[0136] Our results show two means of glucose consumption and two paths from PEP to succinate. The two general routes which E. coli uses to transport and phosphorylate glucose differ in E. coli strains NZN111 and AFP111. One route involves two multienzyme systems collectively termed the **phosphotransferase** system (PTS) which concomitantly transport and phosphorylate glucose to intracellular glucose 6-phosphate by using PEP as a cosubstrate. This route ultimately leads to the formation of both PEP and pyruvate, and the resulting net reaction may be expressed as:

Detail Description Paragraph - DETX (124):

[0137] The one mole of **PEP formed in this reaction is available to PEP** carboxylase to generate OAA, or to pyruvate kinase to generate a second mole of pyruvate and ATP. The one mole committed to pyruvate is not available for direct conversion to OAA. Wild-type E. coli can still grow in the absence of the PTS, and a mutation in the glk gene for glucokinase is necessary to eliminate growth on glucose completely. Thus, a second route for glucose uptake involves glucose transport uncoupled from phosphorylation, a route which generally appears to be insignificant compared to the PTS in wild-type E. coli. The resulting net reaction may be expressed as:

Detail Description Paragraph - DETX (126):

[0138] In this case, two moles of **PEP are available to PEP** carboxylase for OAA formation. Of course, one mole of PEP could form pyruvate via pyruvate kinase with the generation of ATP so that the ultimate equations for the two routes to pyruvate are equivalent. In this study for anerobically grown cells, AFP111 showed markedly greater glucokinase activity than NZN111.

Detail Description Paragraph - DETX (140):

[0148] E. coli AFP111 is a pfl ldhA strain that can grow anaerobically on glucose as the sole carbon source. This strain was has a mutation in the ptsG gene which encodes for an enzyme of the **phosphotransferase** system (PTS). Because of the ptsG mutation, AFP111 relies on glucokinase for glucose uptake. When grown aerobically for biomass generation and then subject to anaerobic conditions (a "dual-phase" fermentation), AFP111 attains succinate yields and productivities of 0.99 and 0.87 g/Lh, respectively (P. Nghiem et al. U.S. Pat. No. 5,869,301).

PGPUB-DOCUMENT-NUMBER: 20030074690

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030074690 A1

TITLE: Novel plastid-targeting nucleic acid sequence, a novel beta-amylase sequence, a stimulus-responsive promoter and uses thereof

PUBLICATION-DATE: April 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kavanagh, Thomas Anthony	Dublin		IE	
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APPL-NO: 10/ 261189

DATE FILED: September 30, 2002

RELATED-US-APPL-DATA:

child 10261189 A1 20020930

parent continuation-of 09375140 19990816 US GRANTED

parent-patent 6489540 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	9817959.1	1998GB-9817959.1	August 19, 1998
GB	9817963.3	1998GB-9817963.3	August 19, 1998
GB	9913014.8	1999GB-9913014.8	March 24, 1999

US-CL-CURRENT: 800/278, 435/204 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2 , 800/284

ABSTRACT:

The invention provides a novel chloroplast targeted novel .beta.-amylase sequence (ct .beta.-amylase), a novel chloroplast targeting nucleic acid sequence and a novel .beta.-amylase sequence. There is also disclosed an inducible promoter which is independently stimulated by light or sugar stimulus. Methods of transforming plants using these sequences are described, as well as transformed plant cells, transformed plants and seed thereof, as well as chimaeric genes containing the sequences.
Modification of starch levels in plants can be achieved, as well as the targeting of genes from the starch biosynthetic or degradative pathways, disease or pest resistance or variation of gene expression due to stimulus are described.

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Summary of Invention Paragraph - BSTX (14):

[0014] The ability to manipulate the amount of starch in the plastids of leaves or storage organs would be of high benefit to various industrial processes which utilise plant starches. For example, in an attempt to increase the starch content of potato tubers, it has been shown previously that when E. coli ADPG PPase glgC16 is overexpressed in transgenic potato tubers, there is an **increase in flux of carbon** into starch but there is only a small increase in net accumulation of starch (Sweetlove et al., 1997). Analysis of enzyme activities in the overexpressing lines showed that, apart from the alteration in ADPG PPase, the activity of amylase, specifically .beta.-amylase was also altered. This data suggests that the accumulation of starch in tubers overexpressing glgC16 protein is prevented by the breakdown of the newly synthesised starch, i.e. the starch is being turned over.

Summary of Invention Paragraph - BSTX (57):

[0057] Advantageously the first transformed plant is a plant having an increased enzyme activity in the starch biosynthetic pathway. An example of an attempt to increase the starch content of a plant is a transgenic potato transformed with the gene for ADPG-PPase, for example glgC16 (see for example, WO 91/19806). The amount of starch increase in such a plant has been relatively small. This first transformed plant is advantageously retransformed with a chimaeric gene for a starch degrading enzyme, suitably comprising, for example, At ct .beta.-amylase. The glgC16 protein is expressed in the first transformed tubers and results in increased ADPG-PPase activity and an **increase in flux of carbon** to starch. Advantageously, the expression of the chimaeric At ct .beta.-amylase gene, or parts thereof, in the retransformed tubers results in down regulation of the ct .beta.-amylase activity, i.e. cosuppression or antisense technology, thus providing for an increase in starch accumulation.

Brief Description of Drawings Paragraph - DRTX (3):

[0069] FIG. 3 shows the diagrammatic representation of the T-DNA of the chimaeric ct .beta.-amylase promoter-GUS genes constructed in Example 3 below, in which NosP represents the nopaline synthase promoter; NosT represents the nopaline synthase terminator; BR is the right border inverted repeat and BL is the left border inverted repeat of the T-DNA of pBI101; NPTII represents the neomycin **phosphotransferase** II coding sequence; GUS represents the .beta.-glucuronidase coding sequence. ct .beta.-amylase promoter fragments are represented by hatched rectangles; the PCR amplified Xho I-Bam HI bridging fragments are represented by black rectangles;

Brief Description of Drawings Paragraph - DRTX (7):

[0073] FIG. 7 shows the plasmid map of binary plasmid pBNP10431 where 35Sp represents the CaMV 35S promoter, ct bamy represents the full length ct .beta.-amylase cDNA, 35St represents the CaMV 35S terminator, RB represents the

right border of the binary vector pBinPlus, colElori represents the colEl origin bacterial replication, RKori represents the oriV origin of replication of the RK2 plasmid, nptIII represents the neomycin **phosphotransferase** gene for bacterial resistance to kanamycin, LB represents the left border sequence of the binary vector, and kan represents the plant neomycin **phosphotransferase** recombinant gene required for plant resistance to kanamycin;

US-PAT-NO: 6663906

DOCUMENT-IDENTIFIER: US 6663906 B2

TITLE: Expression of fructose 1,6 bisphosphate aldolase in transgenic plants

DATE-ISSUED: December 16, 2003

INVENTOR-INFORMATION:

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Cheikh; Nordine	Manchester	MO	N/A	N/A
Kishore; Ganesh M.	Creve Coeur	MO	N/A	N/A

APPL-NO: 10/ 164204

DATE FILED: June 6, 2002

PARENT-CASE:

This application is a divisional of application Ser. No. 09/098,219 filed Jun. 16, 1998, now U.S. Pat. No. 6,441,277, which is based on U.S. provisional application Serial No. 60/049,955 filed Jun. 17, 1997.

US-CL-CURRENT: 426/438

ABSTRACT:

Fructose-1,6-bisphosphate aldolase (FDA) is an enzyme reversibly catalyzing the reaction converting triosephosphate into fructose-1,6-bisphosphate. In the leaf, this enzyme is located in the chloroplast (starch synthesis) and the cytosol (sucrose biosynthesis). Transgenic plants were generated that express the E. coli fda gene in the chloroplast to improve plant yield by increasing leaf starch biosynthetic ability in particular and sucrose production in general. Leaves from plants expressing the fda transgene showed a significantly higher starch accumulation, as compared to control plants expressing the null vector, particularly early in the photoperiod, but had lower leaf sucrose. Transgenic plants also had a significantly higher root mass. Furthermore, transgenic potatoes expressing fda exhibited improved uniformity of solids.

6 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Brief Summary Text - BSTX (17):

First, increasing the expression of the FDA enzyme in the chloroplast would **increase the flow of carbon through the Calvin Cycle and increase** atmospheric carbon assimilation during early photoperiod. This would result in an increase in photosynthetic efficiency and an increase in chloroplast starch production (a leaf carbon storage form degraded during periods when photosynthesis is low or absent). Both of these responses would lead to an increase in sucrose production by the leaf and a net increase in carbon export during a given photoperiod. This increase in source capacity is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Brief Summary Text - BSTX (19):

Third, expression of FDA in sink tissues can show several desirable traits, such as increased amino acid and/or fatty acid pools via **increases in carbon flux** through glycolysis (and thus pyruvate levels) in seeds or other sinks and increased starch levels as result of increased production of glucose 6-phosphate in seeds, roots, stems, and tubers where starch is a major storage nonstructural carbohydrate (reverse glycolysis). This increase in sink strength is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Detailed Description Text - DETX (2):

This invention is directed to a method for producing plant cells and plants demonstrating an increased or improved growth and development, yield, quality, starch storage uniformity, vigor, and/or stress tolerance. The method utilizes a DNA sequence encoding an fda (fructose 1,6 bisphosphate aldolase) gene integrated in the cellular genome of a plant as the result of genetic engineering and causes expression of the FDA enzyme in the transgenic plant so produced. Plants that overexpress the FDA enzyme exhibit **increased carbon flow through the Calvin Cycle and increased** atmospheric carbon assimilation during early photoperiod resulting in an increase in photosynthetic efficiency and an increase in starch production. Thus, such plants exhibit higher levels of sucrose production by the leaf and the ability to achieve a net increase in carbon export during a given photoperiod. This increase in source capacity leads to increased plant growth that in turn generates greater biomass and/or increases the size of the sink and ultimately providing greater yields of the transgenic plant. This greater biomass or increased sink size may be evidenced in different ways or plant parts depending on the particular plant species or growing conditions of the plant overexpressing the FDA enzyme. Thus, increased size resulting from overexpression of FDA may be seen in the seed, fruit, stem, leaf, tuber, bulb or other plant part depending upon the plant species and its dominant sink during a particular growth phase and upon the environmental effects caused by certain growing conditions, e.g. drought, temperature or other stresses. Transgenic plants overexpressing FDA may therefore have increased carbon assimilation, export and storage in plant source and sink organs, which results in growth, yield, and uniformity and quality improvements.

Detailed Description Text - DETX (3):

Plants overexpressing FDA may also exhibit desirable quality traits such as increased production of starch, oils and/or proteins depending upon the plant species overexpressing the FDA. Thus, overexpression of FDA in a particular plant species may affect or **alter the direction of the carbon flux** thereby directing metabolite utilization and storage either to starch production, protein production or oil production via the role of FDA in the glycolysis and gluconeogenesis metabolic pathways.

Detailed Description Text - DETX (36):

A recombinant DNA molecule of the invention typically includes a selectable marker so is that transformed cells can be easily identified and selected from non-transformed cells. Examples of such include, but are not limited to, a neomycin **phosphotransferase** (nptII) gene (Potrykus et al., 1985), which confers kanamycin resistance. Cells expressing the nptII gene can be selected using an appropriate antibiotic such as kanamycin or G418. Other commonly used selectable markers include the bar gene, which confers bialaphos resistance; a mutant EPSP synthase gene (Hinchee et al., 1988), which confers glyphosate resistance; a nitrilase gene, which confers resistance to bromoxynil (Stalker et al., 1988); a mutant acetolactate synthase gene (ALS), which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204, 1985); and a methotrexate resistant DHFR gene (Thillet et al., 1988).

Detailed Description Text - DETX (87):

This increase in sucrose export by fda-expressing leaves is an illustration of an increase in source capacity, very likely due to an **increased carbon flow** through the Calvin Cycle (in response to increased triose-P utilization) and thus an increase in net carbon utilization by the leaf. As seen in Table 2, the increase in sucrose loading in the phloem correlates with the level of fda expression.

Detailed Description Text - DETX (93):

For the cytosolic expression of the fda gene in corn plants, a construct was made in which the fda gene sequence was fused to the backbone of a vector containing the enhanced CaMV 35S promoter (e35S; Kay et al., 1987), the HSP70 intron (U.S. Pat. No. 5,593,874), and the NOS3' polyadenylation sequence (Fraley et al., 1983). This created a NotI cassette [P-e35S/HSP70 intron/fda/NOS3'] that was cloned into the NotI site of pMON30460, a monocot transformation vector, to form the plant transformation vector pMON13925, as shown in FIG. 5. pMON30460 contains an expression cassette for the selectable marker neomycin **phosphotransferase** typeII gene (nptII) [P-35S/NPTII/NOS3'] and a unique NotI site for cloning the gene of interest. The final vector (pMON13925) was constructed so that the gene of interest and the selectable marker gene were cloned in the same orientation. A vector fragment containing the expression cassettes for these gene sequences could be excised from the bacterial selector (Kan) and ori, gel purified, and used for plant transformation.

Detailed Description Text - DETX (97):

Transgenic maize plants transformed with the vectors pMON13925 (described above) or pMON17590 (described above) were produced using microprojectile bombardment, a procedure well-known to the art (Fromm, 1990; Gordon-Kamm et al., 1990; Walters et al., 1992). Embryogenic callus initiated from immature maize embryos was used as a target tissue. Plasmid DNA at 1 mg/mL in TE buffer was precipitated onto M10 tungsten particles using a calcium chloride/spermidine procedure, essentially as described by Klein et al. (1988). In addition to the gene of interest, the plasmids also contained the neomycin **phosphotransferase** II gene (nptII) driven by the 35S promoter from Cauliflower Mosaic Virus. The embryogenic callus target tissue was pretreated on culture medium osmotically buffered with 0.2M mannitol plus 0.2M sorbitol for approximately four hours prior to bombardment (Vain et al., 1993). Tissue was bombarded two times with the DNA-coated tungsten particles using the gunpowder version of the BioRad Particle Delivery System (PDS) 1000 device. Approximately 16 hours following bombardment, the tissue was subcultured onto a medium of the same composition except that it contained no mannitol or sorbitol, and it contained an appropriate aminoglycoside antibiotic, such as G418", to select for those cells that contained and expressed the 35S/nptII gene. Actively growing tissue sectors were transferred to fresh selective medium approximately every 3 weeks. About 3 months after bombardment, plants were regenerated from surviving embryogenic callus essentially as described by Duncan and Widholm (1988).

Detailed Description Text - DETX (109):

A second potato transformation vector was constructed by cloning the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the unique NotI site of pMON23616. pMON23616 is a potato transformation vector containing the nopaline-type T-DNA right border region (Fraley et al., 1985), an expression cassette for the neomycin **phosphotransferase** typeII gene [P-35S/NPTII/NOS3'] (selectable marker), a unique NotI site for cloning the gene expression cassette of interest, and the T-DNA left border region (Barker et al., 1983). Cloning of the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the NotI site of pMON23616 results in the potato transformation vector pMON17581, as shown in FIG. 8. The vector pMON17581 was constructed such that the gene of interest and the selectable marker gene were transcribed in the same direction.

US-PAT-NO: 6624343

DOCUMENT-IDENTIFIER: US 6624343 B1

TITLE: Hexose carrier proteins

DATE-ISSUED: September 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Allen; Stephen M	Wilmington	DE	N/A	N/A
Rafalski; J. Antoni	Wilmington	DE	N/A	N/A

APPL-NO: 09/ 679686

DATE FILED: October 5, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of International Application No. PCT/US99/07561 filed Apr. 7, 1999, now pending, which claims priority benefit of U.S. Provisional Application No. 60/081,131 filed Apr. 9, 1998.

US-CL-CURRENT: 800/278, 435/252.3 , 435/320.1 , 435/410 , 435/419 , 435/6 , 435/69.1 , 530/350 , 530/370 , 536/23.1 , 536/23.6 , 536/24.1 , 800/284 , 800/295

ABSTRACT:

This invention relates to an isolated nucleic acid fragment encoding a hexose carrier protein. The invention also relates to the construction of a chimeric gene encoding all or a portion of the hexose carrier protein, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the hexose carrier protein in a transformed host cell.

10 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (55):

The nucleic acid fragments of the instant invention may be used to create

transgenic plants in which the disclosed corn, rice, sorghum, soybean or wheat hexose carrier proteins are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of **altering the flux of carbon** to various cellular compartments in those cells.

Detailed Description Text - DETX (109):

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) Nature 313:810-812), the hygromycin **phosphotransferase** gene from plasmid pJR225 (from E. coli; Gritz et al. (1983) Gene 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the hexose carrier protein and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

US-PAT-NO: 6593093

DOCUMENT-IDENTIFIER: US 6593093 B1

TITLE: Detection of group a Streptococcus

DATE-ISSUED: July 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Uhl; James R.	Rochester	MN	N/A	N/A
Cockerill; Franklin R.	Rochester	MN	N/A	N/A

APPL-NO: 10/ 081923

DATE FILED: February 20, 2002

US-CL-CURRENT: 435/6

ABSTRACT:

The invention provides methods to detect Group A Streptococcus (GAS) in biological samples using real-time PCR. Primers and probes for the detection of GAS are provided by the invention. Articles of manufacture containing such primers and probes for detecting GAS are further provided by the invention.

35 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Detailed Description Text - DETX (7):

A metabolic pathway chart showing the **phosphoenolpyruvate:phosphotransferase system is available** online. Briefly, the phosphoenolpyruvate:**phosphotransferase** system (pep:pts or pts) is composed of two enzymes, HPr and enzyme I (or EI) encoded by the ptsH and ptsI genes, respectively. Enzyme I is autophosphorylated by phosphoenolpyruvate. Phosphorylated EI then catalyzes the phosphorylation of HPr in the membrane. HPr phosphorylates a sugar-specific enzyme that is translocated across the membrane. Thus, EI and HPr are necessary for sugar translocation. The **phosphotransferase** system is reviewed by, for example, Postma et al. (1993, Microbiol. Rev., 57:543-94) and the pts operon is reviewed by, for example, Vadeboncoeur et al. (2000, J. Mol. Microbiol. Biotechnol., 2:483-90).

Detailed Description Text - DETX (8):

The invention provides methods to detect GAS by amplifying, for example, GAS nucleic acid molecules corresponding to a portion of the ptsI gene encoding enzyme I (EI) of the phosphoenolpyruvate:sugar **phosphotransferase** system. GAS nucleic acid molecules other than those exemplified herein (e.g., other than ptsI) also can be used to detect GAS in a sample and are known to those of skill in the art. Nucleic acid sequences encoding GAS ptsI have been described (see, for example, Ferretti et al., 2001, Proc. Natl. Acad. Sci. USA, 98:4658-63; and GenBank Accession Nos. NC 002737, and AE004092). Specifically, primers and probes to amplify and detect GAS ptsI nucleic acid molecules are provided by the invention.

US-PAT-NO: 6537815

DOCUMENT-IDENTIFIER: US 6537815 B2

TITLE: Method of altering the expression of csrB to modify the properties of a cell

DATE-ISSUED: March 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Romeo; Tony	Burlington	TX	N/A	N/A

APPL-NO: 09/ 736734

DATE FILED: December 13, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 09/112,584, filed Jul. 9, 1998, now U.S. Pat. No. 6,228,638 which claims the benefit of U.S. Provisional Application Ser. No. 60/052,372, filed Jul. 11, 1997, the disclosures of which are incorporated herein by reference in their entirety.

US-CL-CURRENT: 435/471, 435/375

ABSTRACT:

The invention includes the gene csrB, the RNA encoded thereby and methods of use thereof. csrB RNA binds to and antagonizes the ability of CsrA to down-regulate the production of certain metabolic products. This invention is also drawn to methods of using csrB polynucleotides, and combination of csrB polynucleotides and CsrA polypeptides and antibodies that bind to such combinations.

8 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Brief Summary Text - BSTX (14):

Further "metabolic engineering" can lead to even greater yields of desired amino acids or other products. In addition to being an amino acid precursor,

PEP is a precursor of glucose via gluconeogenesis. Glucose is, in turn, a precursor of glycogen. Gluconeogenesis and glycogen synthesis are elevated in csrA mutants and would compete for the synthesis of aromatic amino acids. Therefore, in order to further **increase carbon flow** into the desired products (e.g., amino acids), engineering of gluconeogenesis, glycogen biosynthesis and possibly other pathways is desirable. A mutation in fbp, which encodes fructose-1,6-bisphosphatase, prevents gluconeogenesis from proceeding beyond the synthesis of fructose-1,6-bisphosphate. A mutation in glgC (ADP-glucose pyrophosphorylase) or glgA (glycogen synthase) further blocks residual glucose or glucose derivatives obtained from the media or generated within the cell from being used for glycogen synthesis. Each of these mutations is already known and can be introduced into a cell by methods known in the art. Further enhancement of the synthesis of a single aromatic amino acid can be achieved by introducing mutations which block the synthesis of other amino acids.

Other Reference Publication - OREF (58):

Reizer et al., (1996). "Novel **phosphotransferase**-encoding genes revealed by analysis of the Escherichia coli genome: a chimeric gene encoding an Enzyme I homologue that possesses a putative sensory transduction domain" Gene 181(1-2):103-8.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	4270	carbon near2 (flux or flow)	USPAT; US-PGPUB	2003/12/24 07:56
2	L2	275	1 near4 (modif$8 or alter$8 or increas$8)	USPAT; US-PGPUB	2003/12/24 07:56
3	L3	166	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	USPAT; US-PGPUB	2003/12/24 07:57
4	L4	9	2 and 3	USPAT; US-PGPUB	2003/12/24 07:57
5	L5	6159	phosphotransferase$1 or phospho adj transferase$1	USPAT; US-PGPUB	2003/12/24 08:10
6	L6	36	(2 or 3) and 5	USPAT; US-PGPUB	2003/12/24 08:11
7	L7	40	(2 or 3) same (aromatic or shikimate)	USPAT; US-PGPUB	2003/12/24 08:14

PGPUB-DOCUMENT-NUMBER: 20030203460

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030203460 A1

TITLE: Biocatalytic synthesis of quinic acid and conversion to hydroquinone

PUBLICATION-DATE: October 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Frost, John W.	Okemos	MI	US	
Frost, Karen M.	Okemos	MI	US	

APPL-NO: 10/ 435242

DATE FILED: May 9, 2003

RELATED-US-APPL-DATA:

child 10435242 A1 20030509

parent continuation-of 09427394 19991025 US GRANTED

parent-patent 6600077 US

child 09427394 19991025 US

parent continuation-in-part-of 09240441 19990129 US ABANDONED

US-CL-CURRENT: 435/136, 568/771

ABSTRACT:

A bioengineered synthesis scheme for the production of quinic acid from a carbon source is provided. Methods of producing quinic acid from a carbon source based on the synthesis scheme as well as conversion of quinic acid to hydroquinone are also provided.

RELATED APPLICATIONS

[0001] The present invention is a continuation of U.S. Ser. No. 09/427,394, filed Oct. 25, 1999, which is a continuation-in-part of U.S. Ser. No. 09/240,441, filed Jan. 29, 1999, which is hereby expressly incorporated by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (8):

[0027] In a preferred embodiment, the recombinant E. coli comprises plasmid pKD12.112 carrying aroF.sup.FBR, serA and aroE gene inserts. The aroF.sup.FBR gene insert encodes a mutant 3-deoxy-D-arabino-heptulosonate- -7-phosphate synthase isozyme (a, FIG. 1) insensitive to feedback inhibition by **aromatic** amino acids or other **aromatic** molecules which **increases carbon flow** into the common **aromatic** amino acid biosynthetic pathway. Due to a mutation in the E. coli genomic serA locus required for L-serine biosynthesis, growth in minimal salts medium and plasmid maintenance follows from expression of plasmid-localized serA. The serA insert thus allows microbial growth in minimal salts medium, distinguishing the microbes containing the plasmid from non-plasmid containing microbes. The aroE gene insert encodes a 3-**shikimate** dehydrogenase, increasing the production of quinic acid. Preferably, the aroE gene is from E. coli. More preferably, all the inserted genes are from E. coli, producing a homogenous recombinant E. coli.

PGPUB-DOCUMENT-NUMBER: 20030190752

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190752 A1

TITLE: Escherichia coli csrB gene, RNA encoded thereby, and methods of use thereof

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Romeo, Tony	Burlington	TX	US	

APPL-NO: 10/ 396911

DATE FILED: March 24, 2003

RELATED-US-APPL-DATA:

child 10396911 A1 20030324

parent division-of 09736734 20001213 US GRANTED

parent-patent 6537815 US

child 09736734 20001213 US

parent continuation-of 09112584 19980709 US GRANTED

parent-patent 6228638 US

non-provisional-of-provisional 60052372 19970711 US

US-CL-CURRENT: 435/455, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention includes the gene csrB, the RNA encoded thereby and methods of use thereof. csrB RNA binds to and antagonizes the ability of CsrA to down-regulate the production of certain metabolic products. This invention is also drawn to methods of using csrB polynucleotides, and combination of csrB polynucleotides and CsrA polypeptides and antibodies that bind to such combinations.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to Provisional Application Serial No. 60/052,372, filed Jul. 11, 1997, the disclosure of which is incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (14):

[0014] Further "metabolic engineering" can lead to even greater yields of desired amino acids or other products. In addition to being an amino acid precursor, PEP is a precursor of glucose via gluconeogenesis. Glucose is, in turn, a precursor of glycogen. Gluconeogenesis and glycogen synthesis are elevated in csrA mutants and would compete for the synthesis of **aromatic** amino acids. Therefore, in order to further **increase carbon flow** into the desired products (e.g., amino acids), engineering of gluconeogenesis, glycogen biosynthesis and possibly other pathways is desirable. A mutation in fbp, which encodes fructose-1,6-bisphosphatase, prevents gluconeogenesis from proceeding beyond the synthesis of fructose-1,6-bisphosphate. A mutation in glgC (ADP-glucose pyrophosphorylase) or glgA (glycogen synthase) further blocks residual glucose or glucose derivatives obtained from the media or generated within the cell from being used for glycogen synthesis. Each of these mutations is already known and can be introduced into a cell by methods known in the art. Further enhancement of the synthesis of a single **aromatic** amino acid can be achieved by introducing mutations which block the synthesis of other amino acids.

PGPUB-DOCUMENT-NUMBER: 20030138920

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138920 A1

TITLE: Process for the isolation of polyhydroxy cyclic carboxylic acids

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Malmberg, Mats	Lund		SE	
Westrup, Brita	Lund		SE	

APPL-NO: 10/ 041865

DATE FILED: January 7, 2002

US-CL-CURRENT: 435/136, 424/94.1

ABSTRACT:

This invention is directed to the use of crystallization acids, such as acetic, lactic and propionic acids, to obtain high purity polyhydroxyl cyclic carboxylic acids (PCCA) from low purity aqueous solutions. The preferred PCCA is shikimic acid and the preferred crystallization acid is acetic acid. The method according to the invention is particularly applicable to the isolation of shikimic acid from a fermentation broth.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (51):

[0046] Yet another source of shikimic acid upon which the present invention can be conducted is obtained from fermentations wherein the **carbon flow is increased into the shikimate** pathway by combining metabolic manipulations during the fermentation. Further enhancement of the production of shikimic acid can be achieved through nutrient limitation. For example, the growth of microorganisms can be limited by limiting the cells' availability of **aromatic** amino acids (no exogenous source of **aromatic** amino acids exist in the growth medium). Alternatively, or in combination with the other techniques, shikimic acid production can be enhanced by feeding inorganic phosphate to the microorganisms at a growth limiting rate.

PGPUB-DOCUMENT-NUMBER: 20030134392

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030134392 A1

TITLE: Microorganisms and methods for overproduction of DAHP by cloned Pps gene

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liao, James C.	Los Angeles	CA	US	

APPL-NO: 10/ 289788

DATE FILED: November 7, 2002

RELATED-US-APPL-DATA:

child 10289788 A1 20021107

parent division-of 09440503 19991115 US GRANTED

parent-patent 6489100 US

US-CL-CURRENT: 435/74, 435/252.33 , 435/320.1

ABSTRACT:

Genetic elements comprising expression vectors and a gene coding for phosphoenol pyruvate synthase is utilized to enhance diversion of carbon resources into the common aromatic pathway and pathways branching therefrom. The overexpression of phosphoenol pyruvate synthase increases DAHP production to near theoretical yields.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (15):
[0014] The overproduction of transketolase in tkt transformed cells has been found to provide an **increased flow of carbon** resources into the common **aromatic** pathway relative to carbon resource utilization in whole cells that do not harbor such genetic elements. However, the **increased carbon flux** may be further enhanced by additional manipulation of the host strain.

Summary of Invention Paragraph - BSTX (20):
[0018] The present invention further provides methods of **increasing carbon**

flow into the common **aromatic** pathway of a host cell comprising the step of transforming the host cell with recombinant DNA comprising a pps gene so that Pps is expressed at appropriate point in the metabolic pathways at enhanced levels relative to wild type host cells.

Detail Description Paragraph - DETX (5):

[0033] Besides the use of the pps gene, the present invention also provides for transfer of genetic elements comprising the tkt gene, the gene coding for DAHP synthase (aroF in E. coli), the gene coding for 3-dehydroquinate synthase (aroB in E. coli), or other genes encoding enzymes that catalyze reactions in the common **aromatic** pathway. Such a cell transformation can be achieved by transferring one or more plasmids that contain genes that code for enzymes that **increase the carbon flux** for DAHP synthesis and for subsequent synthesis of other desired cyclic, pre-**aromatic, and aromatic** metabolites. As a result of this transfer of genetic element(s), more carbon enters and moves through the common **aromatic** pathway relative to wild type host cells not containing the genetic elements of the present invention.

Detail Description Paragraph - DETX (6):

[0034] In one embodiment, the present invention comprises a method for **increasing carbon flow** into the common **aromatic** pathway of a host cell by increasing the production of DAHP through the overexpression of Pps at the appropriate point in the common **aromatic** pathway to provide additional PEP at the point where PEP condenses with E4P. **Increasing carbon flow** requires the step of transforming the host cell with recombinant DNA containing a pps gene so that Pps is overexpressed at enhanced levels relative to wild type host cells. DAHP is then produced by fermentation of the transformed cell in a nutrient medium where the DAHP can be extracted from the medium on a batch wise or continuous extraction procedure.

Detail Description Paragraph - DETX (8):

[0036] Another embodiment of the present invention is a method for enhancing a host cell's biosynthetic production of compounds derived from the common **aromatic** pathway. This method involves the step of increasing expression of Pps in the host cell relative to a wild type host cell. The step of increasing expression of Pps can include transferring into the host cell a vector carrying the pps gene. The overexpression of Pps results in forcing **increased carbon flow** into the biosynthesis of DAHP.

Detail Description Paragraph - DETX (12):

[0040] Besides being used in the biosynthesis of DAHP, PEP is used as a phosphate donor in the phosphotransferase system (PTS) which is responsible for glucose uptake. Additionally, PEP can be converted to pyruvate by pyruvate kinases and to oxaloacetate by phosphoenolpyruvate carboxylase. All such competing pathways limit the **availability of PEP** for the biosynthesis of DAHP and all metabolites derived from the common **aromatic** pathway or pathways branching therefrom.

Detail Description Paragraph - DETX (93):

[0119] This example demonstrates that the E. Coli AB2847 is unable to utilize DAHP, and accumulates DAHP in the medium if DAHP synthase is overexpressed. This strain was used as a host for detecting the flux committed to the **aromatic** pathways. Since Draths et al. (Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievense, "Biocatalytic synthesis of aromatics from D-glucose: The role of transketolase," J. Am. Chem. Soc., 1992, 114, 3956-3962) have shown a possible limitation in the production of DAHP by E4P, pATI (containing both aroG.sup.fbr and tktA) was transformed into AB2847 to eliminate the limitation of E4P. To test whether **PEP supply limits DAHP production, PEP** synthase (Pps) was overexpressed in AB2847/pAT1 by transforming plasmid pPS341 into this strain. 20-70 copies of the pps gene were expressed in the host cells. As a control, pPS341 was substituted by pPS341X1, which encodes an inactive, but stable pps gene product. The use of the inactive Pps control allowed discrimination between the effect of Pps activity and that of protein overexpression. AB2847/pAT1/pUHE23-2 and AB2847/pAT1 were also used without any other plasmid as additional controls to identify the effect of the cloning vector, pUHE23-2, on DAHP production.

PGPUB-DOCUMENT-NUMBER: 20030068791

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030068791 A1

TITLE: Manufacture of five-carbon sugars and sugar alcohols

PUBLICATION-DATE: April 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Miasnikov, Andrei	Kantvik		FI	
Ojamo, Heikki	Kirkkonummi		FI	
Povelainen, Mira	Espoo		FI	
Gros, Hakan	Kantvik		FI	
Toivari, Mervi	Espoo		FI	
Richard, Peter	Helsinki		FI	
Ruohonen, Laura	Helsinki		FI	
Koivuranta, Kari	Helsinki		FI	
Londesborough, John	Helsinki		FI	
Aristidou, Aristos	Espoo		FI	
Penttila, Merja	Helsinki		FI	
Plazanet-Menut, Claire	Paris		FR	
Deutscher, Josef	Fontenay Le Fleury		FR	

APPL-NO: 09/ 908744

DATE FILED: July 20, 2001

RELATED-US-APPL-DATA:

child 09908744 A1 20010720

parent continuation-in-part-of 09488581 20000121 US ABANDONED

child 09488581 20000121 US

parent continuation-in-part-of 08790585 19970129 US PENDING

child 08790585 19970129 US

parent continuation-of 08368395 19950103 US GRANTED

parent-patent 5631150 US

child 08368395 19950103 US

parent continuation-of 08110672 19930824 US ABANDONED

child 08110672 19930824 US

parent continuation-in-part-of 07973325 19921105 US ABANDONED

child 09908744 A1 20010720

parent continuation-in-part-of PCT/FI01/00051 20010122 US UNKNOWN

US-CL-CURRENT: 435/158, 435/252.3 , 435/254.2

ABSTRACT:

The invention relates to the methods of manufacturing five-carbon sugars and sugar alcohols as well as other compounds derived from pentose-phosphate pathway from readily available substrates such a hexoses using metabolically engineered microbial hosts.

---------- KWIC --------

Detail Description Paragraph - DETX (11):

[0061] Xylulose-5-P can also be a precursor of several important products including xylulose, which in turn is a precursor for D-lyxose (via mannose-isomerase) and D-xylose (via xylose isomerase). Accordingly, the term "xylulose-5-P derived product" as used herein includes xylulose, D-lyxose and D-xylose, and mixtures of the same, but is not restricted to these examples. For example, a strain in which the genetic modifications results in increased relative amounts of xylulose-5-P can be further genetically modified to result in a strain with improved yields of xylulose-5-P derived products such as xylitol. Similarly, a strain that has been modified to have increased amounts of ribose-5-P or an **increased flux of carbon** to ribose-5-P can be further modified to increase the production of ribose-5-P derived products, and especially the production of nucleotides and riboflavin, or D-erythrose 4-P and products thereof, such as folate, ubiquinone and various **aromatic** amino acids. Similarly, as described herein for products such as sugar alcohols, production of ribose-5-P derived products in a strain accumulating ribose-5-P or having improved flux of carbon to ribose-5-P can be further improved by genetic modification of the subsequence/downstream metabolic reactions leading to such products.

PGPUB-DOCUMENT-NUMBER: 20030059913

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059913 A1

TITLE: Novel Microorganisms with ability to degrade indole and novel enzymes therefrom

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Weyler, Walter	San Francisco	CA	US	
Resnick, Sol M.	Solana Beach	CA	US	

APPL-NO: 09/ 570777

DATE FILED: May 14, 2000

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09570777 A1 20000514

parent continuation-of 08858663 19970519 US ABANDONED

child 08858663 19970519 US

parent continuation-in-part-of 08560729 19951120 US GRANTED

parent-patent 6190892 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
	PCT/US97/16641		September 18, 1997

US-CL-CURRENT: 435/191, 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

There is provided novel indole oxidase activity isolated from P. putida, such indole oxidase activity is believed to be useful in the biosynthetic production of indigo from its precursor indole. Also provided are compositions of matter comprising the indole oxidase and methods for producing such.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser.

No. 08/560,729 filed Nov. 20, 1995 (pending), and which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (14):

[0013] Tryptophan pathway genes useful in securing biosynthetic indole accumulation include a trp operon, isolated from a microorganism as a purified DNA molecule that encodes an enzymatic pathway capable of directing the biosynthesis of L-tryptophan from chorismic acid. (A. J. Pittard (1987) Biosynthesis of **Aromatic** Amino Acids in Escherichia coli and Salmonella typhimurium, F. C. Neidhardt, ed., American Society for Microbiology, publisher, pp. 368-394.) Indole accumulation is enabled by modification of one or more of the pathway's structural elements and/or regulatory regions. This modified trp operon may then be introduced into a suitable host such as a microorganism, plant tissue culture system or other suitable expression system. It should be noted that the term "indole accumulation" does not necessarily indicate that indole actually accumulates intracellularly. Instead, this term can indicate that there is an **increased flux of carbon** to indole and indole is made available as a substrate for intracellular catalytic reactions such as indoxyl formation and other than the formation of L-tryptophan. In the context of this invention, the "accumulated" indole may be consumed in the conversion of indole to indoxyl by an oxygenase such as the **aromatic** dioxygenase NDO, or an **aromatic** monooxygenase such as TMO, or it may actually build up intracellularly and extracellularly, as would be the case when the desired end product is indole or one of its derivatives.

PGPUB-DOCUMENT-NUMBER: 20030054519

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054519 A1

TITLE: MICROORGANISMS WITH ABILITY TO DEGRADE INDOLE AND ENZYMES THEREFROM

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Weyler, Walter	San Francisco	CA	US	
Resnick, Sol M.	Solana Beach	CA	US	

APPL-NO: 09/ 570779

DATE FILED: May 14, 2000

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09570779 A1 20000514

parent continuation-of 08858663 19970519 US ABANDONED

child 08858663 19970519 US

parent continuation-in-part-of 08560729 19951120 US GRANTED

parent-patent 6190892 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US97/16641	1997WO-PCT/US97/16641	September 18, 1997

US-CL-CURRENT: 435/189, 435/252.3 , 435/320.1 , 435/69.1

ABSTRACT:

There is provided novel indole oxidase activity isolated from P. putida, such indole oxidase activity is believed to be useful in the biosynthetic production of indigo from its precursor indole. Also provided are compositions of matter comprising the indole oxidase and methods for producing such.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser.

No. 08/560,729 filed Nov. 20, 1995 (pending), and which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (14):

[0013] Tryptophan pathway genes useful in securing biosynthetic indole accumulation include a trp operon, isolated from a microorganism as a purified DNA molecule that encodes an enzymatic pathway capable of directing the biosynthesis of L-tryptophan from chorismic acid. (A. J. Pittard (1987) Biosynthesis of **Aromatic** Amino Acids in Escherichia coli and Salmonella typhimurium, F. C. Neidhardt, ed., American Society for Microbiology, publisher, pp. 368-394.) Indole accumulation is enabled by modification of one or more of the pathway's structural elements and/or regulatory regions. This modified trp operon may then be introduced into a suitable host such as a microorganism, plant tissue culture system or other suitable expression system. It should be noted that the term "indole accumulation" does not necessarily indicate that indole actually accumulates intracellularly. Instead, this term can indicate that there is an **increased flux of carbon** to indole and indole is made available as a substrate for intracellular catalytic reactions such as indoxyl formation and other than the formation of L-tryptophan. In the context of this invention, the "accumulated" indole may be consumed in the conversion of indole to indoxyl by an oxygenase such as the **aromatic** dioxygenase NDO, or an **aromatic** monooxygenase such as TMO, or it may actually build up intracellularly and extracellularly, as would be the case when the desired end product is indole or one of its derivatives.

US-PAT-NO: 6642031

DOCUMENT-IDENTIFIER: US 6642031 B2

TITLE: Microorganisms with ability to degrade indole and enzymes therefrom

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weyler; Walter	San Francisco	CA	N/A	N/A
Resnick; Sol M.	Solana Beach	CA	N/A	N/A

APPL-NO: 09/ 570779

DATE FILED: May 14, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. application Ser. No. 08/858,663, filed May 19, 1997 now abandoned, which is a continuation application of U.S. application Ser. No. 08/719,381 filed Sep. 25, 1996 now abandoned, which is a continuation-in-part of U.S. application Ser. No. 08/560,729 filed Nov. 20, 1995, now U.S. Pat. No. 6,190,892 and which are incorporated herein by reference in their entirety.

US-CL-CURRENT: 435/71.1, 435/243 , 435/252.1 , 435/253.3 , 435/68.1 , 435/69.1 , 530/350

ABSTRACT:

There is provided novel indole oxidase activity isolated from P. putida, such indole oxidase activity is believed to be useful in the biosynthetic production of indigo from its precursor indole. Also provided are compositions of matter comprising the indole oxidase and methods for producing such.

6 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (14):

Tryptophan pathway genes useful in securing biosynthetic indole accumulation include a trp operon, isolated from a microorganism as a purified DNA molecule that encodes an enzymatic pathway capable of directing the biosynthesis of L-tryptophan from chorismic acid. (A. J. Pittard (1987) Biosynthesis of **Aromatic** Amino Acids in Escherichia coli and Salmonella typhimurium, F. C. Neidhardt, ed., American Society for Microbiology, publisher, pp. 368-394.) Indole accumulation is enabled by modification of one or more of the pathway's structural elements and/or regulatory regions. This modified trp operon may then be introduced into a suitable host such as a microorganism, plant tissue culture system or other suitable expression system. It should be noted that the term "indole accumulation" does not necessarily indicate that indole actually accumulates intracellularly. Instead, this term can indicate that there is an **increased flux of carbon** to indole and indole is made available as a substrate for intracellular catalytic reactions such as indoxyl formation and other than the formation of L-tryptophan. In the context of this invention, the "accumulated" indole may be consumed in the conversion of indole to indoxyl by an oxygenase such as the **aromatic** dioxygenase NDO, or an **aromatic** monooxygenase such as TMO, or it may actually build up intracellularly and extracellularly, as would be the case when the desired end product is indole or one of its derivatives.

US-PAT-NO: 6620602

DOCUMENT-IDENTIFIER: US 6620602 B2

TITLE: Biocatalytic synthesis of quinic acid and conversion to hydroquinone

DATE-ISSUED: September 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Frost; John W.	Okemos	MI	N/A	N/A
Frost; Karen M.	Okemos	MI	N/A	N/A

APPL-NO: 10/ 099429

DATE FILED: March 15, 2002

PARENT-CASE:

RELATED APPLICATIONS

The present invention is a division of U.S. Ser. No. 09/427,394, filed Oct. 25, 1999, which is a continuation-in-part of U.S. Ser. No. 09/240,441, filed Jan. 29, 1999, now abandoned, which are hereby expressly incorporated by reference.

US-CL-CURRENT: 435/136, 435/132 , 435/133 , 435/190 , 435/232 , 435/252.1 , 435/252.2 , 435/252.3 , 435/252.33 , 435/254.1 , 435/254.11 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

A bioengineered synthesis scheme for the production of quinic acid from a carbon source is provided. Methods of producing quinic acid from a carbon source based on the synthesis scheme as well as conversion of quinic acid to hydroquinone are also provided.

30 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Detailed Description Text - DETX (8):

In a preferred embodiment, the recombinant E. coli comprises plasmid

pKD12.112 carrying aroF.sup.FBR, serA and aroE gene inserts. The aroF.sup.FBR gene insert encodes a mutant 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase isozyme (a, FIG. 1) insensitive to feedback inhibition by **aromatic** amino acids or other **aromatic** molecules which **increases carbon flow** into the common **aromatic** amino acid biosynthetic pathway. Due to a mutation in the E. coli genomic serA locus required for L-serine biosynthesis, growth in minimal salts medium and plasmid maintenance follows from expression of plasmid-localized serA. The serA insert thus allows microbial growth in minimal salts medium, distinguishing the microbes containing the plasmid from non-plasmid containing microbes. The aroE gene insert encodes a 3-**shikimate** dehydrogenase, increasing the production of quinic acid. Preferably, the aroE gene is from E. coli. More preferably, all the inserted genes are from E. coli, producing a homogenous recombinant E. coli.

US-PAT-NO: 6613552

DOCUMENT-IDENTIFIER: US 6613552 B1

TITLE: Biocatalytic synthesis of shikimic acid

DATE-ISSUED: September 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Frost; John W.	Okemos	MI	N/A	N/A
Frost; Karen M.	Okemos	MI	N/A	N/A
Knop; David R.	Okemos	MI	N/A	N/A

APPL-NO: 09/ 240440

DATE FILED: January 29, 1999

US-CL-CURRENT: 435/136, 435/132 , 435/146 , 435/252.3 , 435/252.33 , 435/320.1 , 435/471 , 435/488 , 435/69.1 , 536/23.2

ABSTRACT:

A bioengineered synthesis scheme for the production of shikimic acid from a carbon source is provided. Methods of producing shikimic acid from a carbon source based on the synthesis scheme are also provided.

42 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

--------- KWIC ---------

Detailed Description Text - DETX (7):

In a preferred embodiment, the recombinant E. coli comprises plasmid pKD12.112 carrying aroF.sup.FBR, serA and aroE inserts. The aroF.sup.FBR gene insert encodes a mutant 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase isozyme (a, FIG. 1) insensitive to feedback inhibition by **aromatic** amino acids or other **aromatic** molecules which **increases carbon flow** into the common **aromatic** amino acid biosynthetic pathway. Amplified **shikimate** dehydrogenase resulting from expression of aroE compensates for the enzyme's feedback inhibition by shikimic acid. Pittard, J. et al., J. Bacteriol. 92:1070 (1966); Brown, K. D. et al., Biochim. Biophys. Acta. 428:550 (1976). Due to a mutation in the E. coli genomic serA locus required for L-serine biosynthesis, growth in minimal salts medium and plasmid maintenance follows from expression of plasmid-localized serA. The plasmid serA insert thus allows

microbial growth in minimal salts medium, distinguishing the microbes.

US-PAT-NO: 6600077

DOCUMENT-IDENTIFIER: US 6600077 B1

TITLE: Biocatalytic synthesis of quinic acid and conversion to hydroquinone

DATE-ISSUED: July 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Frost; John W.	Okemos	MI	N/A	N/A
Frost; Karen M.	Okemos	MI	N/A	N/A

APPL-NO: 09/ 427394

DATE FILED: October 25, 1999

PARENT-CASE:

RELATED APPLICATIONS

The present invention is a continuation-in-part of U.S. Ser. No. 09/240,441, filed Jan. 29, 1999, now abandoned, which is hereby expressly incorporated by reference.

US-CL-CURRENT: 568/749, 435/133 , 435/155 , 435/156 , 552/291 , 552/293 , 568/322 , 568/716 , 568/811

ABSTRACT:

A bioengineered synthesis scheme for the production of quinic acid from a carbon source is provided. Methods of producing quinic acid from a carbon source based on the synthesis scheme as well as conversion of quinic acid to hydroquinone are also provided.

9 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Detailed Description Text - DETX (8):

In a preferred embodiment, the recombinant E. coli comprises plasmid pKD12.112 carrying aroF.sup.FBR, serA and aroE gene inserts. The aroF.sup.FBR gene insert encodes a mutant 3-deoxy-D-arabino-heptulosonate-7-phosphate

synthase isozyme (a, FIG. 1) insensitive to feedback inhibition by **aromatic** amino acids or other **aromatic** molecules which **increases carbon flow** into the *common* ***aromatic*** *amino acid biosynthetic pathway. Due to a mutation in the* E. coli genomic serA locus required for L-serine biosynthesis, growth in minimal salts medium and plasmid maintenance follows from expression of *plasmid-localized serA. The serA insert thus allows microbial growth in* minimal salts medium, distinguishing the microbes containing the plasmid from non-plasmid containing microbes. The aroE gene insert encodes a 3-**shikimate** dehydrogenase, increasing the production of quinic acid. Preferably, the aroE gene is from E. coli. More preferably, all the inserted genes are from E. coli, producing a homogenous recombinant E. coli.

US-PAT-NO: 6537815

DOCUMENT-IDENTIFIER: US 6537815 B2

TITLE: Method of altering the expression of csrB to modify the properties of a cell

DATE-ISSUED: March 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Romeo; Tony	Burlington	TX	N/A	N/A

APPL-NO: 09/ 736734

DATE FILED: December 13, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 09/112,584, filed Jul. 9, 1998, now U.S. Pat. No. 6,228,638 which claims the benefit of U.S. Provisional Application Ser. No. 60/052,372, filed Jul. 11, 1997, the disclosures of which are incorporated herein by reference in their entirety.

US-CL-CURRENT: 435/471, 435/375

ABSTRACT:

The invention includes the gene csrB, the RNA encoded thereby and methods of use thereof. csrB RNA binds to and antagonizes the ability of CsrA to down-regulate the production of certain metabolic products. This invention is also drawn to methods of using csrB polynucleotides, and combination of csrB polynucleotides and CsrA polypeptides and antibodies that bind to such combinations.

8 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Brief Summary Text - BSTX (14):

Further "metabolic engineering" can lead to even greater yields of desired amino acids or other products. In addition to being an amino acid precursor,

PEP is a precursor of glucose via gluconeogenesis. Glucose is, in turn, a precursor of glycogen. Gluconeogenesis and glycogen synthesis are elevated in csrA mutants and would compete for the synthesis of **aromatic** amino acids. Therefore, in order to further **increase carbon flow** into the desired products (e.g., amino acids), engineering of gluconeogenesis, glycogen biosynthesis and possibly other pathways is desirable. A mutation in fbp, which encodes fructose-1,6-bisphosphatase, prevents gluconeogenesis from proceeding beyond the synthesis of fructose-1,6-bisphosphate. A mutation in glgC (ADP-glucose pyrophosphorylase) or glgA (glycogen synthase) further blocks residual glucose or glucose derivatives obtained from the media or generated within the cell from being used for glycogen synthesis. Each of these mutations is already known and can be introduced into a cell by methods known in the art. Further enhancement of the synthesis of a single **aromatic** amino acid can be achieved by introducing mutations which block the synthesis of other amino acids.